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Abstract

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The Sustainable Development Goals (SDG) aim to eliminate preventable child mortality by 2030. Despite the notable improvement in child survival in sub-Saharan Africa in the past decade overall, progress has been heterogeneous, with some areas experiencing persistently high under-5 mortality. In these settings, unprecedented reductions in child mortality will be required to achieve the SDG target.

Biannual azithromycin distribution has been shown to reduce mortality in children under 5, particularly in high mortality settings. As this simple intervention has been implemented in trachoma programs globally for decades, it presents an effective, feasible approach to addressing the child mortality burden. Before implementation of this intervention is considered, however, questions remain about the optimal program design. A major risk of this intervention is the potential to select for antimicrobial resistance, and so implementers must weigh the intervention's benefits against the risks. In response, this dissertation aims to inform the design of a program to distribute azithromycin to improve child survival while addressing the risk of increasing antimicrobial resistance.

This dissertation uses data collected during trachoma and mortality studies assessing azithromycin distribution. Azithromycin has been distributed in community-based programs to control trachoma in endemic areas since the 1990s, and numerous studies have been conducted to evaluate the impact of these programs on a range of outcomes, including antimicrobial resistance. An important gap in understanding of this intervention's impact in the broader context of child survival is knowledge of its potential to select for antimicrobial resistance. Chapter 1 systematically reviews the literature on macrolide resistance after azithromycin distribution in trachoma programs to characterize the risk of resistance in a variety of settings. To mitigate the risk of antimicrobial resistance, future programs could target vulnerable subgroups of the population, such as malnourished children. This approach would limit the amount of antibiotics distributed, theoretically reducing the risk of selecting for resistance, while focusing the intervention on those at the highest risk of mortality. Chapter 2 explores the potential impact of targeting underweight children by using data from a cluster-randomized trial of the efficacy of azithromycin distribution in reducing child mortality to evaluate whether the effect of the

intervention differs by nutritional status. Finally, existing estimates of the effect of this intervention on mortality are based on intention-to-treat estimates, which might not capture the full population-level impact of the intervention, particularly in real-world settings that might experience varying patterns of intervention uptake. Using data from the same trial, Chapter 3 estimates the effect of the intervention among eligible treated children to estimate the per protocol effect and among eligible untreated children to determine the presence of spillover effects from treated to untreated subgroups. Overall, these findings contribute to the global discussion on approaches to improving child survival and could inform the direction of both future research in this area and implementation of this intervention.

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For my partner, Ryan Charles Hoffman.

II. ACKNOWLEDGEMENTS

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III. INTRODUCTION

Global burden of child mortality

The burden of child mortality is an important indicator of population health. Targets for reducing mortality in children are crucial components of the development agenda for improving health globally. The Millennium Development Goals (MDGs) aimed to reduce under-5 mortality by two-thirds by 2015.[1] In the era of the Sustainable Development Goals (SDGs), targeting the burden of under-5 mortality remains a priority, with SDG 3.2 aiming to reduce under-5 mortality to 25 deaths per 1,000 livebirths or lower by 2030.[2]

Under-5 mortality has declined considerably since the launch of the MDGs in 1990. Between 2000 and 2016, the global mortality rate for children under 5 decreased from 69.4 deaths per 1,000 live births to 38.4 deaths per 1,000 live births.[3] However, amid this impressive progress there remains substantial variation in the absolute burden of under-5 mortality and in the rates of decline at national and subnational levels.[4] For example, only 57 of 195 countries met or exceeded the 4.4% annualized rate of decline needed to achieve the MDG target for child mortality.[3]

Countries in sub-Saharan Africa continue to have among the highest rates of under-5 mortality, with 28% of global under-5 deaths in 2016 occurring in West Africa and 16% in East Africa.[3] Moreover, striking heterogeneity exists in mortality rates and rates of decline across sub-Saharan African countries. Some areas, like parts of Botswana and Ethiopia, are well poised to reach the SDG target by 2030 or earlier.[4] Areas in central and western Africa, however, will need to achieve annual reductions in under-5 mortality rates of 8.8% or greater in order to reach the target.[4] Realizing these unprecedented rates of decline will require the implementation of highly effective, feasible interventions.

Effects of the mass distribution of azithromycin

The mass distribution of azithromycin is one intervention with the potential to achieve rates of decline of this magnitude. To control the leading infectious cause of blindness, the World Health Organization (WHO) recommends 3-5 years of annual mass distribution of azithromycin in communities with > 10% prevalence of active trachoma among children 1-9 years old.[5] Since the 1990s, trachoma programs have distributed more than 900 million doses of azithromycin in trachoma-endemic areas,[6] representing vast global experience in the implementation of this intervention. These distributions have been shown to reduce the prevalence of infection with *Chlamydia trachomatis*.[7-15] Some settings have even achieved elimination of infection, with mathematical models indicating elimination is possible with antibiotics even in the most severely affected settings.[13,16-19]

Multiple studies on the impact of mass distribution of azithromycin have identified a number of inadvertent beneficial effects of this intervention on outcomes other than trachoma. Some studies have found reductions in the burden of common infectious causes of mortality in children, including reductions in the prevalence of respiratory tract infections, diarrhea, and malaria for 3-6 months after treatment.[20-26] Studies have identified direct effects on mortality as well. A cohort

study and a cluster-randomized trial conducted in a trachoma-endemic area of Ethiopia suggested that communities treated with azithromycin might experience up to a 50% reduction in all-cause mortality in children 1-9 years old compared to untreated communities or children.[27,28]

MORDOR (*Macrolides Oraux pour Réduire les Décès avec un Oeil sur la Résistance* or "oral macrolides to reduce death with an eye on resistance") was a cluster-randomized trial designed to determine the effect of the mass distribution of oral azithromycin on child mortality (Bill and Melinda Gates Foundation, OP1032340).[29] Communities in Malawi, Niger, and Tanzania were randomized to receive biannual oral azithromycin or placebo targeted to children 1-59 months old over a two-year period. A census was conducted biannually to monitor vital status and collect data on the primary outcome, all-cause mortality. To increase the generalizability of results beyond trachoma-endemic areas, the trial was conducted in geographically diverse areas in sub-Saharan Africa that were not eligible for trachoma programs.

MORDOR I randomized 1,624 communities to receive azithromycin or placebo and included 1,512 communities in the primary outcome analysis.[29] Overall, mortality was 13.5% lower in children 1-59 months in communities receiving azithromycin compared to placebo (95% CI 6.7% to 19.8%, $P<0.001$).[29] Among the three countries, Niger had the highest baseline mortality rates (27.5 deaths per 1,000 person-years) and experienced the greatest reduction in mortality in communities receiving azithromycin compared to placebo (18.1% lower, 95% CI 10.0% to 25.5,% $P<0.001$).[29] Further subgroup analyses revealed that the strongest effects were seen in the youngest children (1 to 5 months old), with mortality 24.9% lower in this age group in communities receiving azithromycin compared to placebo (95% CI 10.6% to 37.0%, $P=0.001$).[29] A longer-term follow-up of the trial found continued efficacy of this intervention in a third year of intervention.[30] Although the exact mechanism of effect remains unclear, evidence suggests that azithromycin distributions reduce the burden of respiratory infections, diarrhea, and malaria,[31] common causes of under-5 mortality in sub-Saharan Africa.[3]

The mass distribution of azithromycin is not without risks. Individually, azithromycin is well tolerated in children, though it may cause gastrointestinal side effects such as diarrhea or abdominal pain.[32] Some studies have found associations between azithromycin and the risk of death from cardiac complications in adults, though results of such studies are inconsistent and this relationship has not been identified in children.[33-36] In addition, azithromycin has been linked to infantile hypertrophic pyloric stenosis (IHPS) in neonates.[37-39] In the MORDOR I trial, no differences were identified in adverse events between azithromycin and placebo arms, either through passive or active surveillance.[29,40] To avoid the risk of IHPS in neonates, MORDOR I excluded children younger than 1 month of age.

At the population level, increased selection pressure from mass distributions of azithromycin could result in the emergence of antimicrobial resistance (AMR), threatening the effective prevention and treatment of a range of infectious diseases.[41] Mass azithromycin distribution could lead to selection of macrolide-resistant *Chlamydia trachomatis*, reducing the efficacy of the current stronghold intervention in trachoma control. Moreover, these distributions could lead to resistance to macrolides and other classes of antibiotics in other pathogens as well, including *Streptococcus pneumoniae* and gram-negative *Enterobacteriaceae*. In the current era of antimicrobial stewardship and increasing global concern over the spread of AMR, the potential

for this intervention to select for resistance in macrolides and other classes of antibiotics is a significant concern. After two years of biannual distributions, the MORDOR I trial found increased resistance to macrolides in azithromycin compared to placebo-treated communities with no resistance found in other classes of antibiotics.[42]

Optimal program design

Before implementing mass distribution of azithromycin to improve child survival, important questions remain about the ideal program design to balance the benefits and potential risks of the intervention. In particular, the risk of selecting for AMR has given many potential implementers caution. Characterization of the magnitude, duration, and heterogeneity of this risk across settings is important in guiding program implementation. Although one recent mortality trial has provided direct, short-term evidence on the selection for AMR after biannual azithromycin for child survival,[42] myriad earlier studies have examined AMR after azithromycin distributed in trachoma programs in multiple settings. Chapter 1 of this dissertation reviews and synthesizes all available evidence on AMR following azithromycin distributed in trachoma programs to provide a broader characterization of this risk to guide implementers considering this intervention.

One approach to mitigating the risk of selecting for AMR is to target smaller, high-risk subgroups of the under-5 population, which would limit the antibiotics distributed and so theoretically reduce the spread of resistance, while still focusing the intervention on those facing the highest risk of mortality. Subgroups defined by nutritional status are of particular interest, as undernutrition is associated with an increased risk of infectious disease and related mortality.[43] Moreover, antibiotics are routinely used in the management of uncomplicated severe acute malnutrition and have been shown to reduce mortality,[44,45] although results have been mixed in different settings.[46] If the effect of azithromycin on mortality seen in MORDOR is driven by its effect in malnourished children, then this vulnerable subgroup could be targeted by programs rather than treating the entire population of children. Chapter 2 examines whether biannual azithromycin distribution has a differential effect on mortality in subgroups of children defined by nutritional status.

The preferred analytic approach for randomized controlled trials (RCTs) is intention-to-treat (ITT),[47] in which individuals are classified according to assigned treatment, regardless of compliance with assigned treatment. Proper randomization will, on average, balance confounders between treatment arms, ensuring an unbiased comparison of treatment effect; ITT analyses preserve these benefits of randomization. ITT analyses thus provide valid measures of the causal *effect of treatment assignment*. With perfect intervention uptake, ITT analyses also provide valid estimates of *the effect of treatment* itself. In RCTs with imperfect uptake, however, the analysis and interpretation of the estimate of the causal effect of treatment (as opposed to treatment assignment) depends on the pattern of uptake. In a placebo-controlled trial like MORDOR with imperfect uptake, ITT analyses are typically conservative and the effect of treatment assignment is biased towards the null.[47] Estimates of effect among those actually receiving treatment are useful for implementers in settings with varying uptake patterns, though naïve per protocol analyses that fail to properly adjust for compliance-related confounding may be biased.[48]

Analyses that estimate spillover effects are also important complements to estimates of direct treatment effects. Spillovers are a component of the population-level impact of an intervention in which individuals who did not directly receive the intervention may benefit through physical or social proximity to recipients of the intervention.[49]. If spillover effects are present, studies that only estimate overall treatment effects may under- or over-estimate the impact of the intervention, depending on whether or not the spillover effects are in the same direction of the treatment effect. Mass drug administration has been shown to have strong positive spillover effects from treated participants to untreated participants in some settings.[50] Understanding the effect of the mass distribution of azithromycin on mortality among those not receiving treatment will allow for a more comprehensive assessment of the population-level impact of this intervention. Chapter 3 assesses the impact of biannual distribution of azithromycin on eligible treated children to estimate the per protocol effect and among eligible untreated children to estimate spillover effects.

Complex considerations underlie the question of whether to use azithromycin distribution to improve child survival. Given the feasibility and efficacy of this intervention, some argue that withholding such a program would be unethical from both a humanitarian and human rights perspective, particularly in the context of global agendas like the SDGs.[51] With the potential risks, especially of increasing the burden of AMR and its impact on future population health, the question remains how to weigh the potential benefits against the risks in decisions of implementation. Overall, this dissertation aims to contribute to the global discussion on the use of azithromycin distribution to reduce the burden of child mortality in high mortality settings.

1. Chapter 1. Antimicrobial resistance following mass azithromycin distribution for trachoma: a systematic review

1.1 Abstract

Mass azithromycin distribution is a core component of trachoma programs and may reduce under-5 mortality in some settings. This systematic review synthesizes evidence on the emergence of antimicrobial resistance after mass azithromycin distribution. Electronic databases were searched for eligible publications through September 13, 2017. Studies on community-wide distribution of oral azithromycin for trachoma that assessed macrolide resistance in any organism were included. The prevalence of resistance was extracted from published reports and unpublished data were requested from authors of included studies. Of 196 studies identified, 19 met inclusion criteria (12 assessed *Streptococcus pneumoniae*). Macrolide resistance after azithromycin was reported in three of the five organisms studied. The lack of resistance in *Chlamydia trachomatis* suggests that azithromycin may remain effective for trachoma programs, though evidence is limited. As mass azithromycin distribution continues for trachoma and is considered for other indications, ongoing monitoring of antimicrobial resistance will be required.

1.2 Introduction

The World Health Organization recommends 3-5 years of annual mass azithromycin distribution (single directly observed oral dose at 20 mg/kg) to control trachoma in communities with >10% prevalence of trachomatous inflammation—follicular (TF) among children aged 1-9 years.[5] Mass azithromycin reduces the prevalence of trachoma and can even eliminate infection with ocular strains of *Chlamydia trachomatis*, trachoma's causative agent.[8,9,11,13,16-18] Mass azithromycin distribution for trachoma may also reduce the burden of other childhood infectious diseases, including respiratory tract infections, diarrhea, and malaria.[20-25,52] Studies have even found reductions in all-cause mortality after mass azithromycin.[27,28,53] A recent large, cluster-randomized trial demonstrated that biannual azithromycin distribution reduced under-5 mortality by 14% compared to placebo.[53] The results of this study have initiated a global conversation on the role of mass azithromycin distribution in areas with high child mortality rates, and may lead to the inclusion of mass azithromycin distribution in child survival programs.

Though mass azithromycin distribution reduces trachoma prevalence and improves child survival, it may select for macrolide resistance in the target (*C. trachomatis*) and non-target organisms. Resistance selection could decrease the effectiveness of azithromycin for trachoma over time. Moreover, bystander selection could affect other potentially pathogenic organisms and impact treatment for various conditions.[54,55] For example, in some settings, macrolides are recommended as first line agents in the treatment of community-acquired pneumonia.[56] Selection of resistance in *Streptococcus pneumoniae* from azithromycin distribution for trachoma could therefore detrimentally affect management of this condition. A systematic review published in 2015 assessed resistance in *S. pneumoniae* after mass distribution of azithromycin in articles reported through 2013.[57] The authors found a correlation between baseline prevalence of macrolide resistance and resistance after treatment. In communities in which baseline *S. pneumoniae* resistance to azithromycin was low, mass distribution of azithromycin for trachoma

increased resistance temporarily, with the prevalence of resistance subsequently declining after the cessation of treatment. In communities in which baseline resistance or frequency of azithromycin administration was high, resistance remained high after treatment.

This study updates the previous systematic review with articles published since 2013 on *S. pneumoniae* resistance to azithromycin and expand the scope to include reports of azithromycin resistance in other organisms. The objective was to synthesize the existing evidence on the prevalence of antimicrobial resistance following mass azithromycin distribution for trachoma and define future research priorities.

1.3 Methods

Search strategy and selection criteria

We conducted a systematic review of the literature without language restrictions for all years possible. We searched Cochrane Library, Embase, MEDLINE, and Web of Science for studies published from database inception through July 10, 2017. A second search was conducted on June 14, 2018 to capture recently published literature. Conference abstracts from the Association for Research in Vision and Ophthalmology (ARVO), the American Society for Tropical Medicine and Hygiene (ASTMH), the International Symposium on Human Chlamydial Infections (ISHCI), and the World Health Organization's Trachoma Scientific Informal Workshop (TSIW) were searched as well. For ARVO and ASTMH, we searched all abstracts available online. For ISHCI, paper and electronic versions of the conference proceedings were searched for all meetings since 1998. For TSIW, we obtained electronic versions of abstracts for all meetings from the conference organizers. Grey literature was searched through two online databases, the Grey Literature Report and Open Grey. Hand-searching was conducted by reviewing reference lists from all included articles. In addition, all first and corresponding authors of included articles were contacted by email to identify additional unpublished data from studies meeting our inclusion criteria.

For all electronic searches, search terms included azithromycin, resistance, and trachoma. Variations of the following search string were used when appropriate: (azithromycin OR Zithromax) AND resistan* AND trachoma. Full search strategy details are provided in Supplementary Material.

Titles and abstracts were screened for relevance for all retrieved citations. Full articles were reviewed for eligibility after passing the initial screening. Two independent reviewers (KSO and EGD) conducted the title and abstract screening and the full-text review, and discrepancies were adjudicated by a third reviewer (CEO).

We included studies on the community-wide distribution of oral azithromycin for trachoma that included measurement of the prevalence of carriage and macrolide sensitivity for any organism. Studies on the use of azithromycin for purposes other than the prevention and treatment of trachoma were excluded, as were mathematical modeling studies, surveillance reports, and review articles.

Outcomes, data extraction, and quality

Data were extracted from included full articles independently by KSO and EGD. Discrepancies were adjudicated by KSO. Data extraction and adjudication were conducted using REDCap electronic data capture tools hosted at the University of California, San Francisco.[58] Outcomes of interest included the prevalence of carriage and the prevalence of macrolide resistance in all organisms before and after mass distribution of oral azithromycin for trachoma. Other variables extracted included geographic location of study, study design, sample size, treatment coverage, frequency and duration of mass administration of azithromycin, study subject sampling method, specimen sampling method, and resistance assessment method. Risk of bias was assessed using the Cochrane Collaboration's risk of bias tool for randomized studies and the ROBINS-I tool for non-randomized studies.[59,60] The rating scales for these tools were slightly modified to be more similar to each other, with all included studies rated as having High risk, Moderate risk, Low risk, or Unclear risk of bias in each category. Risk of bias was assessed only for the elements of the studies pertaining to estimating prevalence of carriage and resistance. For studies reporting secondary analyses, we attempted to obtain the original publication and incorporated information from the original publication into the qualitative synthesis.

Data synthesis and statistical analysis plan

A qualitative synthesis of included studies was conducted by organism. The pre-specified analysis plan indicated meta-analysis of prevalence of resistance to azithromycin and carriage by organism, overall across included studies and by pre-specified subgroups. Ultimately, the original meta-analysis was not performed as intended given the wide variation in study design, frequency of azithromycin distribution, and the timing of follow-up across studies. We instead identified studies on *S. pneumoniae* that included a 6-month follow-up time point and graphed the prevalence of macrolide resistance at 6 months after the final treatment by treatment frequency. We calculated the average resistance by treatment frequency, weighted by number of included communities. The study protocol was registered at PROSPERO (CRD42017071592).

1.4 Results

A total of 213 records were identified through database searching and other sources (Figure 1). After removing duplicates, 126 records were included in the title and abstract screening. Twenty-seven records met the criteria for full review after the initial screening, including 26 full-text articles and one study unpublished at the time of the search. The full review resulted in exclusion of eight records for the following reasons: six presented data already included in other full-text articles, one did not assess community-wide distribution of azithromycin for trachoma, and one assessed serotype distribution in *S. pneumoniae* isolates. Nineteen articles were included in the qualitative synthesis, including 12 articles on *S. pneumoniae* and seven articles on other organisms.

Streptococcus pneumoniae

Characteristics of studies on macrolide resistance in Streptococcus pneumoniae

The characteristics of included studies on macrolide resistance in *S. pneumoniae* are shown in Table 1. Of the 12 included studies, three were conducted in Nepal,[21,61,62] three in Ethiopia,[63-65] three in Tanzania,[66-68] and one each in Australia,[69] the Gambia,[70] and Niger.[71] The number of communities included ranged from 1-32, with the majority of studies including fewer than 10 communities (median 6 communities). None of the studies reported that communities had undergone mass distribution of azithromycin prior to the distribution whose effect on *S. pneumoniae resistance* profiles was being assessed. Five studies targeted treatment to children,[21,61,62,64,69] six treated all non-pregnant individuals greater than 6 months or 1 year of age,[63,65-68,70] and one study assessed both mass treatment and treatment targeted to children.[71] Seven studies reported the number of individuals in the study population.[63-66,69-71] The number of individuals who received treatment ranged from 221 to 5619 (median 2765).

The frequency of azithromycin distribution varied across studies: a single treatment (five studies),[21,61,66,68,69] annual treatment for 3-4 years (three studies),[62,65,67] biannual treatment for 3 years (one study),[63] and quarterly treatment for 1 year (one study).[64] Two studies compared different treatment frequencies.[70,71] Overall treatment coverage was reported in eight studies and ranged from 59% to 91% (median 86%). Five studies included an untreated control group,[62-65,68] and six studies included a baseline assessment of the prevalence of azithromycin resistance before treatment.[21,64,66,68,69,71]

Follow-up assessments ranged from 2 weeks to 4 years after the final treatment. All studies sampled *S. pneumoniae* in children, either randomly selecting within a specified age range or including all children in a given age range. One study also assessed randomly selected individuals 15 years or older.[70] Eleven studies assessed nasopharyngeal samples,[21,61-65,67-71] and one study assessed oropharyngeal samples.[66] Phenotypic resistance of *S. pneumoniae* to azithromycin and/or erythromycin was assessed using disk diffusion and/or minimum inhibitory concentration (MIC) in 11 studies.[21,61-70] Two studies also used targeted PCR to test for common genetic resistance determinants associated with macrolide resistance, *ermB* and/or *mefA/E*,[64,66] and one study only assessed genotypic resistance.[71] Among the 11 phenotypic resistance studies, resistance status was determined by MIC value via E-test strips (AB Biodisk, Sweden and USA) in four studies,[61,64,66,69] and broth dilution Sensititre MIC plates (Trek Diagnostics Inc,. USA) in three studies.[62,63,65] One study used broth dilution MIC testing but did not specify a commercial product.[21] Three studies used Kirby-Bauer disk diffusion to determine resistance status and E-test (AB Biodisk, Sweden or Biomerieux, Marcy l'Etoile, France) to assess MIC values among isolates classified as resistant.[67,68,70] Nine of the these 11 studies defined breakpoints explicitly or by referencing the manufacturer's instructions, the National Committee for Clinical Laboratory Standards,[72] or the Clinical and Laboratory Standards Institute.[73] The remaining two studies did not include a reference.[61,70]

Outcomes of studies on azithromycin resistance in Streptococcus pneumoniae

Figure 2 displays carriage of *S. pneumoniae* for all follow-up time points by treatment frequency studied. Among the six studies that conducted baseline assessments before distribution of azithromycin, prevalence of carriage of *S. pneumoniae* ranged from 10·7% to 85·0% (median 55·9%) before the first treatment.[21,64,66,68,69,71] Among the nine studies that conducted a follow-up visit 6 months after the final treatment, prevalence of carriage ranged from 6·6% to 89·3% (median 84·0%) 6 months after the last treatment.[21,62,63,65,66,68-71] Six studies conducted follow-up visits at more than two time points. Of these, five saw transient decreases in carriage shortly after the final distribution of azithromycin, with returns to initial carriage prevalence over time.[21,63,66,68-70] Prevalence of carriage remained below 52% in four studies at all time points, including in two different treatment frequency groups in one study.[66-68,70]

Figure 3 displays the prevalence of macrolide resistance in *S. pneumoniae* isolates for all follow-up times points by treatment frequency studied. Among the six studies that conducted baseline assessments before distribution of azithromycin, prevalence of resistance ranged from 0·0% to 35·8% (median 1·0%) before the first treatment.[21,64,66,68,69,71] Among the nine studies that conducted follow-up visits 6 months after the final treatment, prevalence of resistance ranged from 0·0% to 81·9% (median 3·1%) 6 months after the last treatment,[21,62,63,65,66,68-71] Of the six studies with more than two follow-up visits, three saw transient increases in prevalence of resistance, with decreases in resistance to near baseline levels over time;[63,69,70] one study saw increases in prevalence at all time points;[68] one saw no resistance at the first two time points and a small increase in resistance at the final time point;[66] and one study saw no resistance at any time point.[21] In the study of genotypic resistance, prevalence of genetic determinants of resistance was identical in annual and biannual treatment arms at baseline (median 20%, IQR 10% to 40%).[71] After 2 years of mass distributions, resistance increased in both groups, to a median of 40% (IQR 20% to 40%) in the annual group and 60% (IQR 50% to 80%) in the biannually treated group (P<0·001).

Figure 4 shows both the prevalence of resistance by treatment frequency for individual studies with a 6-month follow-up and the weighted average resistance across studies by treatment frequency. Of the eight studies included, four gave a single treatment, three gave three annual treatments, and one gave six biannual treatments. The weighted average of resistance 6 months after the final treatment was 42·0%, 53·4%, and 76·8% for single, annual, and biannual treatment frequencies, respectively.

Other organisms

Table 2 summarizes the characteristics of the eight studies included reporting macrolide resistance in organisms other than *S. pneumoniae*.[25,67,74-79] Two studies assessed resistance in *Staphylococcus aureus,*[67,79], three in *Chlamydia trachomatis*,[74-76] three in *Escherichia coli*,[67,77,78] and one in *Plasmodium falciparum.*[25] All studies were conducted in sub-Saharan Africa and treated non-pregnant individuals greater than 6 months or 1 year of age. Five studies reported treatment coverage, which ranged from 89% to 94% (median 91%).[25,74,77-79] Table 3 presents the study outcomes and results for these organisms.

Characteristics and outcomes of studies on macrolide resistance in Staphylococcus aureus

Two studies on resistance in *S. aureus* were included in this review.[67,79] A repeated cross-sectional study in the Gambia compared three annual distributions to a single distribution in eight communities, with follow-up conducted at 1 month and 6 months after the annual distributions and 30 months after the single distribution.[79] This study included neither a baseline assessment nor an untreated control group. The other included study used a cross-sectional design in Tanzania to assess resistance 48 months after four annual distributions in 32 communities and included neither a baseline assessment nor an untreated control group.[67]

The Gambia study sampled 415 individuals from the annually treated communities and 400 individuals from the communities receiving a single distribution, including all children <15 years old and a random sample of adults ≥15 years old.[79] The Tanzania study included 1,047 randomly selected children 1-5 years old.[67] Resistance was assessed with culture and disk diffusion in the Gambia study, and with Kirby-Bauer disk diffusion and Etest in the Tanzania study. Both studies demonstrated macrolide resistance after treatment with azithromycin. The longitudinal study in the Gambia reported a transient increase in resistance after three annual distributions of azithromycin, with resistance increasing from 8·9% at 1 month after treatment, to 34·1% 3 months after treatment, then decreasing to 7·3% 6 months after treatment.[79] The final prevalence of resistance at 6 months after annual distributions was significantly higher than 30 months after a single distribution (7·3% vs. 1·6%; aOR 5·2, 95% CI 1·5 to 18·3, P=0·010).[79]

Characteristics and outcomes of studies on macrolide resistance in Chlamydia trachomatis

Of the 3 included studies on *C. trachomatis,* two were cohort studies conducted in Tanzania,[74,76] and one was a cross-sectional study conducted in Ethiopia.[75] One study distributed a single treatment in one community,[74] one distributed four annual treatments to 32 communities,[76] and another distributed four biannual treatments to 24 communities.[75] One study included an untreated control group,[75] and two studies included a baseline assessment,[74,76] but none of the studies included both. Follow-up was conducted at two months after the final treatment in two studies,[74,76] and 18 months after the final treatment in one study.[75]

To assess resistance, one study sampled 174 individuals with trachoma,[74] one study sampled 552 children 1-5 years old,[75] and one study sampled 354 children <10 years old.[76] Non-standard microbiological protocols were used to assess resistance in *C. trachomatis* in all studies. None of the studies on *C. trachomatis* found evidence of clinically significant azithromycin resistance when comparing groups before and after treatment or treated and untreated groups.

Characteristics and outcomes of studies on macrolide resistance in Escherichia coli

The three included studies on *E. coli* were conducted in Tanzania.[67,77,78] Two studies examined the same population of four treated communities included in a cohort study,[77,78] and the third study used a cross-sectional design in 32 communities.[67] The two studies from the same population assessed resistance at baseline, 1, 3, and 6 months after a single distribution of azithromycin and included an untreated control group.[77,78] The other study assessed resistance 48

months after four annual distributions of azithromycin and included neither a baseline assessment nor an untreated control group.[67]

The two studies from the same population each included 160 children <3 years old, with one of the studies also including an additional sample of children reporting diarrhea.[77,78] The other study included 1,048 randomly-selected children 1-5 years old.[67] Resistance was assessed with Etest in two studies, and Kirby-Bauer disk diffusion in the third. Each of the three studies on *E. coli* found macrolide resistance after treatment. One of the cohort studies reported a short-term increase in macrolide resistant *E. coli* isolates immediately after the single treatment followed by decreasing resistance over 6 months, with the final assessment at 6 months showing macrolide resistance greater than baseline levels.[77] At all time points post-treatment, treated communities in this study had an increased odds of carriage of macrolide resistant isolates compared to untreated communities (1 month aOR 11·2, 95% CI 7·1 to 17·6, P<0·001; 3 month aOR 10·6, 95% CI 3·8 to 29·9, P<0·001; 6 month aOR 4·8, 95% CI 1·5 to 14·9, P<0·001).[77]

Characteristics and outcomes of studies on macrolide resistance in Plasmodium falciparum

The one included study on *P. falciparum* was conducted on a sample from the same cohort study in Tanzania that included measurement of resistance in *S. pneumoniae* and *E. coli*.[25] This study treated four communities with a single distribution of azithromycin and assessed resistance in the treated communities and an untreated control group at baseline, 1, 3, 4, and 6 months after treatment. The investigators randomly selected children <5 years old and their parents, including 1,045 samples in the resistance assessment. Resistance was assessed using targeted PCR. The study found a 73% reduction in malaria infection one month after a treatment when comparing treated to untreated groups (95% CI 43% to 89%). The difference between groups waned over time, and no evidence of azithromycin resistance was identified.

Risk of bias

Supplementary Table 1 displays the results of the risk of bias assessment. Of the 19 studies included in this review, two were randomized controlled trials.[64,71] One trial compared resistance in communities treated with mass azithromycin to untreated communities;[64] the other compared groups treated with different frequencies of mass azithromycin distribution.[71] Of the remaining 17 studies, seven were not designed to attribute causal effects to the intervention: five used uncontrolled before-and-after designs,[61,66,69,74,76] and two did not include any comparison group.[65,67] Five studies used a cohort design and attempted to control for confounding,[25,68,70,77,78] and five studies did not include enough information to assess risk of bias from confounding.[21,62,63,75,79] No study reported masking participants and personnel, and five studies reported masking outcome assessors.[63-65,71,75] Risk of attrition bias was low for nine studies;[21,63-66,70,71,74,79] the other ten studies did not provide enough information on follow-up to assess risk.[61,62,67-69,75-78,80] The two trials had a low risk of bias from selective reporting since outcomes were pre-specified.[64,71] The other studies did not provide enough information to fully assess risk of bias in this category, though these papers generally reported the same types of outcomes for all available time points, indicating the likelihood of selective reporting is relatively low.

1.5 Discussion

This systematic review documents selection for macrolide resistance in three of the five studied organisms following mass azithromycin distribution for the elimination of trachoma as a public health problem. A previous review summarized the evidence on macrolide resistance in *S. pneumoniae* after mass azithromycin distribution from 8 articles published before 2013.[57] Here, we updated this earlier review to include literature available through June 2018 and sought unpublished data, enabling us to include 50% more data on *S. pneumoniae* with 12 studies. We further expanded on the previous review by widening our inclusion criteria to include any organism on which resistance after mass azithromycin distribution had been published. Finally, as the results of the MORDOR trial spur discussion on the role of the mass distribution of azithromycin in child survival, we focus our synthesis of the available evidence not only on emergent antimicrobial resistance, but on outlining research priorities for future work on the effects of mass azithromycin distributions.

Three studies evaluating the effect of mass azithromycin distribution found no evidence of selection for macrolide resistance in the target organism *C. trachomatis*.[74-76] Mass azithromycin distribution is very effective in reducing the prevalence of the ocular strains of *C. trachomatis* that cause trachoma.[7] Given concerns about the potential for azithromycin's reduced effectiveness for trachoma elimination in the face of increasing resistance, the lack of macrolide resistance in *C.* trachomatis found in these studies is encouraging. However, as the number of doses of azithromycin distributed increases each year, continued vigilance will be required to monitor for emergence of macrolide resistance in *C. trachomatis*.

The most commonly-studied organism was *S. pneumoniae*, with 12 studies reporting resistance in *S. pneumoniae* isolates after mass azithromycin distribution for trachoma.[21,61-71] *S. pneumoniae* is an important commensal organism that colonizes the nasopharynx and can cause pneumonia. Overall, these studies demonstrated an increase in macrolide resistance in *S. pneumoniae* immediately after treatment, which appears to dissipate with time since last treatment. Five studies included an untreated control arm, demonstrating substantially more resistance in *S. pneumoniae* in communities that received azithromycin than those that did not, indicating that the increase is likely due to the azithromycin intervention rather than secular trends.[62-65,68] Although heterogeneity in study design, setting, population, treatment frequency, and follow-up time precluded formal meta-analysis, trends in the included studies suggested that increasing treatment frequency (e.g., single, annual, and biannual) increased selection for macrolide resistance in *S. pneumoniae*. Studies and programs that consider greater frequencies of azithromycin distribution should consider the potential for increased selection for macrolide resistance.

Some evidence of selection for macrolide resistance following mass azithromycin distribution was noted in other organisms, including *E. coli* and *S. aureus.* Enterotoxigenic *E. coli* (ETEC) is a major cause of childhood diarrhea, although macrolides are not typically used in the treatment of ETEC. No studies reported assessment of resistance in *Campylobacter spp*, a common cause of childhood diarrhea for which azithromycin is first-line therapy.[81] In mass azithromycin programs for yaws eradication, emergence of azithromycin-resistant *Treponema pallidum* has recently been reported.[82] Assessment of additional potentially pathogenic organisms in areas

with mass azithromycin distribution will be important to fully understand the impact of mass azithromycin distributions on emergence of macrolide resistance.

We found that the majority of studies evaluating resistance selection following mass azithromycin distribution for trachoma focused on Gram-positive organisms, including *S. pneumoniae* and *S. aureus*. Azithromycin remains an important line of treatment for several pathogenic Gram-negative bacteria, including *Neisseria gonorrhoeae*, *Salmonella spp*, and *Shigella spp*. *N. gonorrhoeae* has developed resistance to many available antimicrobials. The Centers for Disease Control currently recommends dual therapy for gonorrhea treatment with ceftriaxone plus azithromycin.[83] However, transmission of azithromycin-resistant strains of *N. gonorrhoeae* has been reported.[84] Previous work has reported a non-significant decrease in the prevalence of *N. gonorrhea* in women following mass azithromycin distribution for trachoma control,[85] but the effect of the mass distribution of azithromycin on resistance selection in *N. gonorrhoeae* has not been well studied. Typhoid and non-typhoidal invasive salmonella infections are major causes of morbidity. Increasing resistance to fluoroquinolones and third-generation cephalosporins has been reported, and azithromycin is commonly used for enteric fever.[86-89] The loss of azithromycin for treatment of enteric fever would represent a significant global health challenge. Furthermore, azithromycin is first-line treatment for shigellosis, a major cause of childhood diarrhea.[90] A major research priority is to understand the effect of mass azithromycin distribution in these and other Gram-negative organisms.

Some evidence indicates that resistance prevalence may decrease after cessation of antibiotic pressure.[63,69,70,77,79] The development of macrolide resistance may result in a fitness cost, which could explain this observed reduction.[91-94] Minimum inhibitory concentrations of *Shigella* spp and *Salmonella* spp are relatively high, and the mechanism of acquisition and transfer of resistance in Gram-negative organisms differs from that of Gram-positive organisms.[95,96] Differences in mechanism of acquisition of resistance may affect how reversible resistance is once selection pressure is removed.[91] For example, the use of azithromycin for the treatment of sexually transmitted infections in men who have sex with men in high-income settings has selected for azithromycin-resistant *Shigella* spp, which is transmitted even among individuals who have not previously been treated with azithromycin.[97] Future research in Gram-negative organisms should also evaluate trends in resistance following removal of azithromycin selection pressure.

Given continued mass azithromycin treatments for trachoma and the potential role of azithromycin in child survival programs,[53] these results underscore several important research priorities. First, we recommend continued longitudinal surveillance of multiple organisms, including both short- and long-term assessments. Short-term assessments yield information about the immediate impact of azithromycin distributions, while long-term assessments will provide evidence of long-term effects following cessation of azithromycin selection pressure. As trachoma is eliminated in some geographic regions and mass azithromycin distributions are stopped, monitoring for several years after cessation of treatment will provide important data on long-term effects of mass azithromycin distribution. Second, assessment of selection for resistance after multiple and increasing mass azithromycin distributions, particularly for *C. trachomatis*, may yield important insight into the impact of many years of annual mass treatment. In some districts of Ethiopia, a decade of annual mass azithromycin distribution has

not consistently led to elimination of infection.[98] Whether resistance contributes to persistent infection in some geographic regions or clusters is unknown. Third, we recommend research continue to include susceptibility testing on antimicrobials to which increased resistance may have particularly deleterious population-level effects. Macrolide use has been associated with increased *S. pneumoniae* resistance to other antimicrobial agents in some studies.[99-101] Here, we focused solely on macrolide resistance, though many studies included in this review also examined resistance to other key antibiotics such as penicillin. Fourth, we recommend adequately powered randomized study designs with baseline assessments and masked laboratory personnel to better understand whether changes in selection for resistant organisms are due to secular trends (e.g., increased community antibiotic consumption) or periodic azithromycin treatments.

1.6 Conclusions

Although there has been enormous success in controlling ocular *C. trachomatis* infection with mass azithromycin distribution, available evidence suggests that these distributions select for macrolide resistance in some potentially pathogenic organisms and there may be a dose response with increasing frequency of distributions. The limited available evidence suggests that when antibiotic selection pressure is removed, the prevalence of resistance may return to baseline levels over time, though most studies followed populations for 6 months or less and results were mixed in studies with shorter follow-up periods. As azithromycin distribution programs are continued for trachoma and potentially for child survival, continued monitoring of resistance in multiple organisms will be required to ensure any unintended consequences of mass azithromycin distribution can be identified and mitigated.

1.7 Figures and Tables

Figure 1. Systematic review flow diagram: records in each stage of the review. Flow of records through the systematic review process depicted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[102]

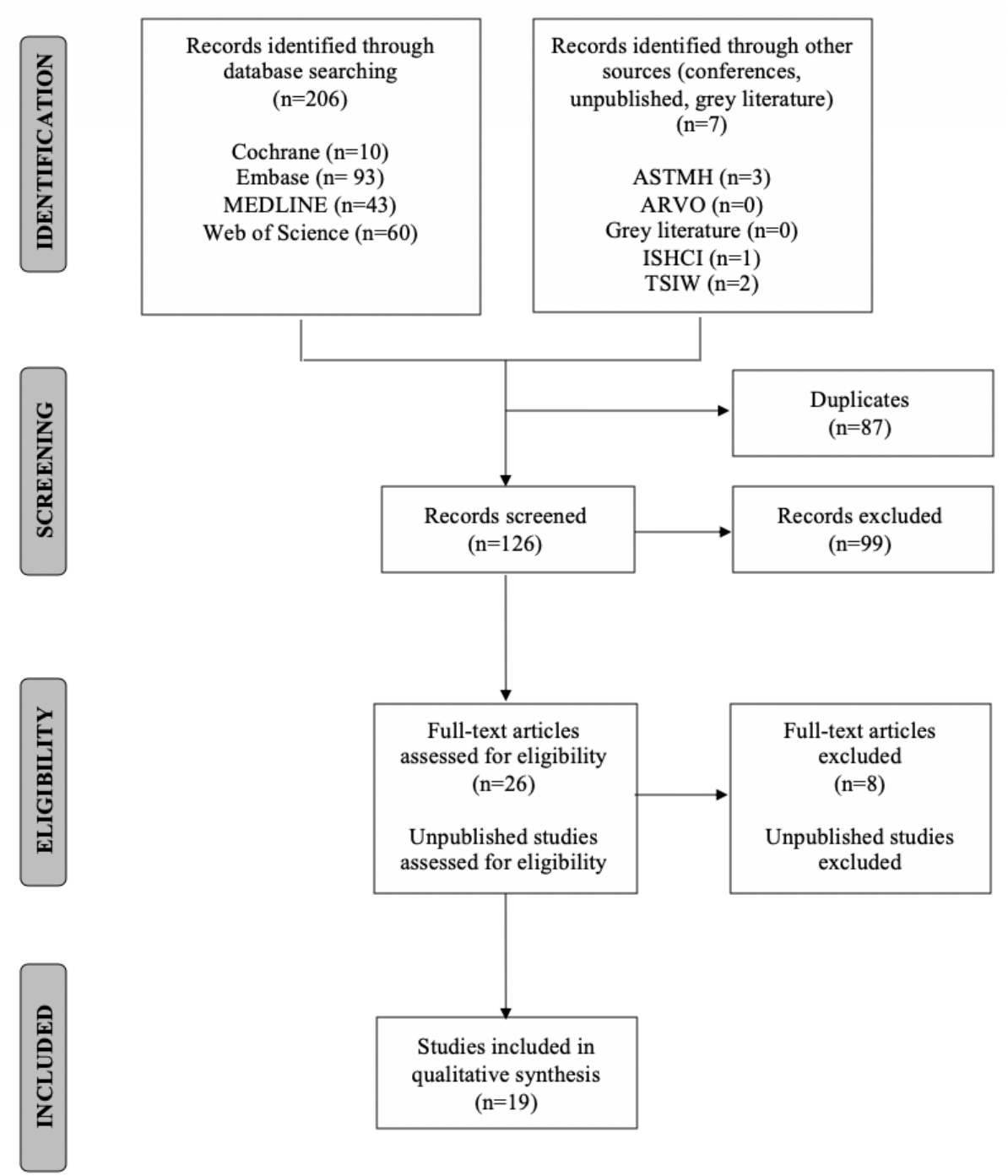

Figure 2. Prevalence of carriage of *Streptococcus pneumoniae* over time among groups treated with azithromycin in included studies. Prevalence of carriage by the number of days after the last distribution of azithromycin is shown for all time points assessed in the 12 different azithromycin-treated groups studied in the 11 unique studies that used microbiological methods to assess phenotypic resistance.

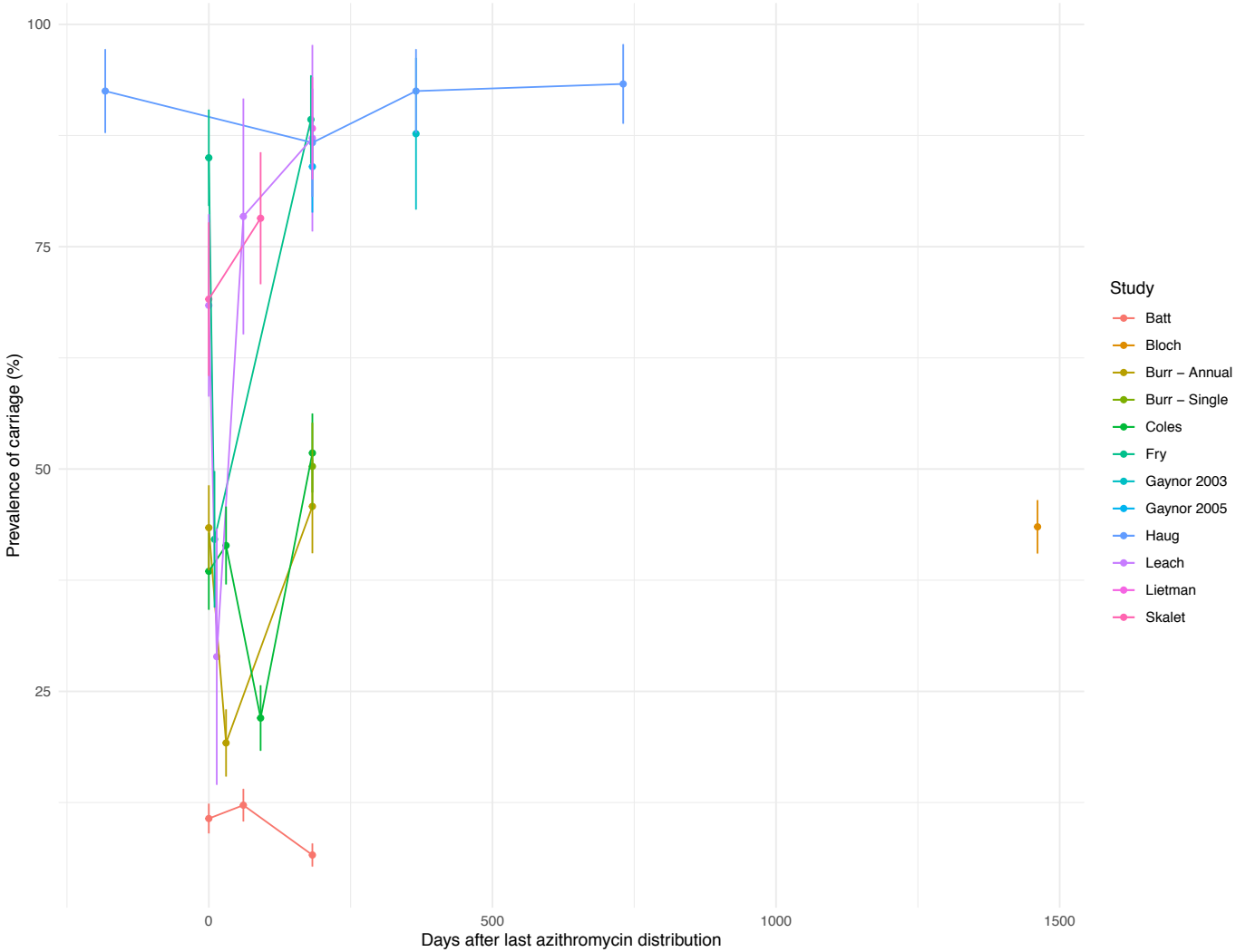

Figure 3. Prevalence of macrolide resistance in *Streptococcus pneumoniae* over time among groups treated with azithromycin in included studies. Prevalence of resistance by the number of days after the last distribution of azithromycin is shown for all time points assessed in the 12 different azithromycin-treated groups studied in the 11 unique studies that used microbiological methods to assess phenotypic resistance.

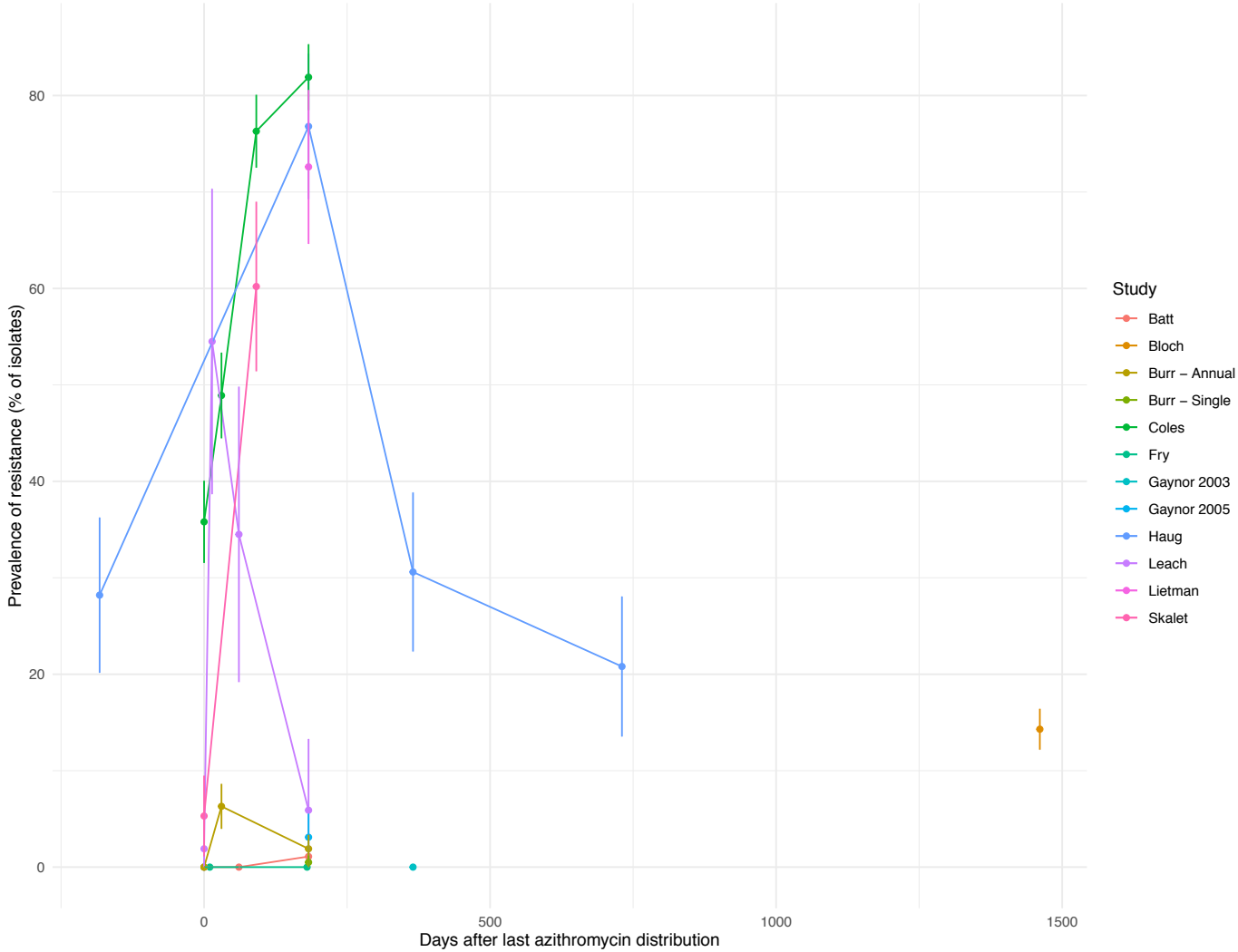

Figure 4. Macrolide resistance in *Streptococcus pneumoniae* 6 months after treatment by treatment frequency. The figure shows the prevalence of resistance reported in individual studies 6 months after the final mass azithromycin distribution by treatment frequency. In addition, a weighted average of resistance by treatment frequency is shown. Each study was weighted by the number of communities included in the study and the average reported resistance was calculated for each treatment frequency.

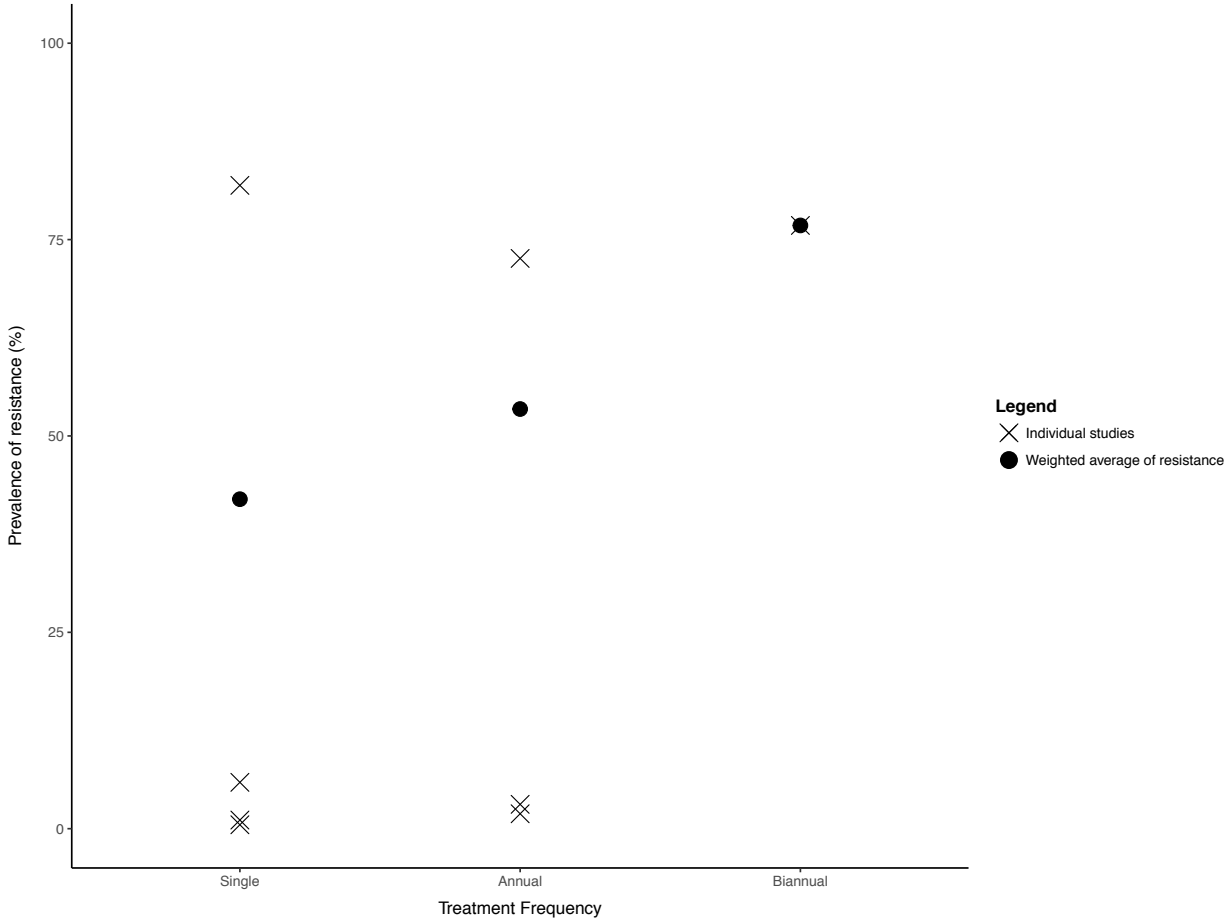

Table 1. Characteristics of included studies on macrolide resistance in *Streptococcus pneumoniae*.

Study	Country	Design	Communities treated[1]	Treatment population	Treatment coverage, % (individuals treated/total treatment population)*	Untreated control	Treatment frequency	Baseline	Follow-up†	Sample population (sample size at first collection in treated group)	Resistance testing
Leach, 1997[1]	Australia	Longitudinal (single group)	1	Children <15 years old with trachoma and household contacts <15 years old	59% (130/221)	No	Single	Yes	2 weeks, 2 months, 6 months	Children <15 years old with trachoma (79)	Etest
Fry, 2002[2]	Nepal	Repeated cross-sectional	3	Children 1-10 years old	NR	No	Single	Yes	10 days, 6 months	Randomly selected children 1-10 years old (167)	Broth dilution MIC
Gaynor, 2003[3]	Nepal	Cross-sectional	1	Children 1-10 years old with trachoma and household contacts	NR	No	Single	No	12 months	Randomly selected children 1-10 years old (57)	E-test
Batt, 2003[4]	Tanzania	Repeated cross-sectional	1	Non-pregnant residents >1 year old	85% (4782/5619)	No	Single	Yes	2 months, 6 months	All children ≤7 years old (1315)	Etest
Gaynor, 2005[5]	Nepal	Cross-sectional	1	Children 1-10 years old	80% (NR)	Yes	Annualx3	No	6 months	All children 1-7 years old (194)	Sensititre

Haug, 2010[6]	Ethiopia	Repeated cross-sectional‡	8	Non-pregnant residents >1 year old	90% (2488/2765)	Yes	Biannualx6	No	6 months, 12 months, 24 months	Randomly selected children 1-5 years old (120)	Sensititre
Skalet, 2010[7]	Ethiopia	Randomized controlled trial	12	Children 1-10 years old	74% (3547/4764)	Yes	Quarterlyx4	Yes	3 months	Randomly selected children <10 years old (110)	Etest
Coles, 2013[8]	Tanzania	Longitudinal (cohort)	4	Non-pregnant residents ≥6 months old	90% (NR)	Yes	Single	Yes	1 month, 3 months, 6 months	Randomly selected children <5 years old (486)	Kirby-Bauer disk diffusion and Etest
Burr, 2014[9]	The Gambia	Repeated cross-sectional‡	8	Non-pregnant residents ≥6 months old	Annual: 89% (715/799) Single: 91% (1019/1124)	No	Annualx3 Single	No	Annual: 0 months 1 month, 6 months Single: 30 months	All children < 15 years old and randomly selected individuals ≥15 years old (annual: 415; single: 400)	Disk diffusion and Etest
Bloch, 2017[10]	Tanzania	Cross-sectional	32	Non-pregnant residents ≥6 months old	NR	No	Annualx4	No	48 months	Randomly selected children 1-59 months old (1047)	Kirby-Bauer disk diffusion and Etest
Keenan, 2018[11]	Niger	Randomized controlled trial	24	Annual: Non-pregnant residents >6 months old Biannual: Children 6 months to 12 years old	Annual: 86% (2508/2916) Biannual: 82% (2556/3132)	No	Annualx3 Biannualx6	Yes	Annual: 12 months Biannual: 6 months	Randomly selected children 0-5 years old (annual: 180; biannual: 168)	Targeted PCR (ermB and mefA/E)

Lietman, NA[12]	Ethiopia	Cross-sectional‡	8	Non-pregnant residents >1 year old	87% (2302/2645)	Yes	Annualx3	No	6 months	Randomly selected children 1-5 years old (120)	Sensititre

MIC = Minimum Inhibitory Concentration, NR = Not reported, NA = Not available (unpublished)
*Treatment at baseline; where possible, numbers exclude tetracycline-treated subjects
†Follow-up time points presented as time after final treatment
‡Subset of communities included in a cluster-randomized trial

Table 2. Characteristics of included studies on macrolide resistance in organisms other than *Streptococcus pneumoniae.*

Study	Country	Design	Communities treated[1]	Treatment population	Treatment coverage, % (individuals treated/total treatment population)*	Untreated control	Treatment frequency	Baseline	Follow-up†	Sample population (sample size at first collection in treated group)	Resistance Testing
Chlamydia trachomatis											
Solomon, 2005[13]	Tanzania	Longitudinal (single group)	1	Non-pregnant residents ≥1 year old	94% (916/978)	No	Single	Yes	2 months	All individuals with trachoma (174)	Culture
Hong, 2009[14]	Ethiopia	Cross-sectional	24	Non-pregnant residents ≥1 year old	NR	Yes	Biannualx4	No	18 months	All children 1-5 years old (552)	Culture
West, 2014[15]	Tanzania	Longitudinal (single group)	32	Non-pregnant residents ≥6 months old	NR	No	Annualx4	Yes	2 months	All children <10 years old with trachoma (359)	Culture
Escherichia coli											
Seidman, 2014[16‡]	Tanzania	Longitudinal (cohort)	4	Non-pregnant residents ≥6 months old	91% (NR)	Yes	Single	Yes	1 month, 3 months, 6 months	40 children <3 years old per community (160)	Etest
Seidman, 2016[17‡]	Tanzania	Longitudinal (cohort)	4	Non-pregnant residents ≥6 months old	91% (NR)	Yes	Single	Yes	1 month, 3 months, 6 months	40 children <3 years old per community (160) plus all children reporting diarrheal symptoms (NR)	Kirby-Bauer disk diffusion

Bloch, 2017[10]	Tanzania	Cross-sectional	32	Non-pregnant residents ≥6 months old	NR	No	Annualx4	No	48 months	Randomly selected children 1 month-5 years old (1048)	Etest
Plasmodium falciparum											
Schachterle, 2014[18]	Tanzania	Longitudinal (cohort)	4	All residents	91% (6252/6894)	Yes	Single	Yes	1 month, 3 months, 4 months, 6 months	Randomly selected parent-child pairs, children <5 years old (1045)	Targeted PCR
Staphylococcus aureus											
Bojang, 2017[19]	The Gambia	Repeated cross-sectional[§]	8	Non-pregnant residents ≥6 months old	Annual: 89% (715/799) Single: 90% (1019/1129)	No	Annualx3 Single	No	Annual: 1 month 6 months Single: 30 months	All children <15 years old and randomly selected individuals ≥15 years old (annual: 415; single: 400)	Culture and disk diffusion
Bloch, 2017[10]	Tanzania	Cross-sectional	32	Non-pregnant residents ≥6 months old	NR	No	Annualx4	No	48 months	Randomly selected children 1 month-5 years old (1047)	Kirby-Bauer disk diffusion and Etest

NR = Not reported

*Treatment at baseline; where possible, numbers exclude tetracycline-treated subjects

†Follow-up time points presented as time after final treatment

‡Similar study population

§Subset of communities included in a cluster-randomized trial

Table 3. Reported results from included studies on macrolide resistance in organisms other than *Streptococcus pneumoniae.*

Study	Outcomes	Outcome Details	Results*	Summary and Conclusions
Chlamydia trachomatis				
Solomon, 2005[13]	MCC	Mean MCC at baseline and 2 months MCC for individuals with positive culture at both time points	Prevalence of culture positive (treated) Baseline: 82% (46/56) 2 months: 89% (8/9) Mean MCC (treated) Baseline: 0·6 μg/mL 2 months: 1·0 μg/mL	The authors found no evidence of an increase in azithromycin resistance after a single mass distribution
Hong, 2009[14]	MIC MCC	MIC and MCC by serotype and treatment group 18 months after MDA	Prevalence of culture positive (treated) 18 months: 70% (7/10) Prevalence of culture positive (untreated) 18 months: 60% (6/10) Mean MIC (treated) 18 months: 0·5 μg/mL Mean MIC (untreated) 18 months: 0·4 μg/mL Mean MCC (treated) 18 months: 0·5 μg/mL Mean MCC (untreated) 18 months: 0·6 μg/mL	MICs and MCCs were comparable between biannually treated communities and untreated communities (MIC: $P = 0{\cdot}76$, MCC; $P = 1{\cdot}00$) The authors found no evidence of an increase azithromycin resistance when comparing biannually treated communities to untreated communities
West, 2014[15]	MIC MBC	MIC and MBC by individual for baseline and 2 months	Mean MIC (treated) Baseline: 0·26 μg/mL 2 months: 0·20 μg/mL Mean MBC (treated) Baseline: 0·27 μg/mL 2 months: 0·26 μg/mL	The authors found no evidence of resistance to azithromycin in any sample

Escherichia coli				
Seidman, 2014[16†]	Prevalence of carriage Prevalence of resistance Odds Ratios for resistance by treatment	Prevalence of carriage and resistance to azithromycin and erythromycin at baseline, 1 month, 3 months, and 6 months by treatment group and compared in logistic regression models	<u>Prevalence of resistance (treated)</u> Baseline: 10% (NR/300) 1 month: 44% (NR/347) 3 months: 30% (NR/347) 6 months: 23% (NR/191) <u>Prevalence of resistance (untreated)</u> Baseline:19% (NR/205) 1 month: 14% (NR/325) 3 months: 10% (NR/324) 6 months: 12% (NR/118)	The prevalence of carriage of macrolide-resistant *E. coli* increased after a single mass distribution of azithromycin, then decreased over time, remaining above baseline levels 6 months after treatment Compared to untreated communities, communities treated with azithromycin experienced significantly increased odds of carriage of macrolide resistant isolates over time (1 month aOR 11·21, 95% CI 7·13 to 17·63, *P*<0·001; 3 month aOR 10·64, 95% CI 3·79 to 29·92, *P*<0·001; 6 month aOR 4·76, 95% CI 1·52 to 14·90, *P*<0·001)
Seidman, 2016[17†]	Pathogenic status of isolates Prevalence of resistance Odds Ratios for resistance by treatment	Prevalence of resistance by pathogenic status and compared by treatment group in logistic regression models	<u>Prevalence of resistance (pathogenic):</u> 35% (243/687) <u>Prevalence of resistance (non-pathogenic):</u> 27% (491/1805)	The prevalence of macrolide resistance in pathogenic *E. coli* was significantly higher than the prevalence in non-pathogenic *E. coli* Azithromycin treatment was significantly associated with increased odds of carriage of macrolide-resistant isolates (aOR 3·64, 95% CI 2·38 to 5·78, *P*<0·001)
Bloch, 2017[10]	Prevalence of carriage Prevalence of resistance MIC	Prevalence of carriage, prevalence of resistance, and MIC ranges 48 months after MDA	<u>Prevalence of carriage (treated)</u> 48 months: 62% (646/1047) <u>Prevalence of resistance (treated)</u>: 48 months: 17% (107/644) <u>Prevalence of MICs ≥32 µg/mL</u> 48 months: 83% (86/103)	The authors found a moderate amount of resistance 4 years after the mass distribution of azithromycin

Plasmodium falciparum				
Schachterle, 2014[18]	Prevalence of infection Sequencing of *P. falciparum* ribosomal L4 protein	Prevalence of infection at baseline, 1 month, 3 months, 4 months, and 6 months after MDA by treatment group Sequencing of full-length P falciparum ribosomal L4 protein for samples from 12 patients	<u>Prevalence of infection (treated)</u> Baseline: 6% (53/854) 1 month: 2% (14/851) 3 months: 2% (15/715) 4 months: 1% (5/637) 6 months: 1% (4/625) <u>Prevalence of infection (untreated)</u> Baseline: 6% (54/894) 1 month: 5% (37/779) 3 months: 3% (17/670) 4 months: 2% (8/531) 6 months: 1% (4/593) <u>Sequencing</u> No evidence of resistance	A single mass distribution of azithromycin resulted in a short-term reduction in the prevalence of malaria without selecting for azithromycin resistance
Staphylococcus aureus				
Bojang, 2017[19]	Prevalence of carriage Prevalence of resistance	Prevalence of carriage and resistance at all time points in both arms	<u>Prevalence of carriage (annual)</u> 1 month: 25% (102/414) 3 months: 39% (161/417) 6 months: 9% (30/343) <u>Prevalence of carriage (single)</u> 30 months: 7% (25/375) <u>Prevalence of resistance (annual)</u> 1 month: 9% (37/414) 3 months: 34% (142/417) 6 months: 7% (25/343) <u>Prevalence of resistance (single)</u> 30 months: 2% (6/375)	The prevalence of carriage at the final survey was similar between treatment arms (aOR 1·47, 95%CI 0·72 to 3·00, *P*=0·286) The prevalence of resistance increased over time in annually treated communities, with a significant difference between treatment groups found at the final survey (aOR 5·22, 95% CI 1·49 to 18·34, *P*=0·010)
Bloch, 2017[10]	Prevalence of carriage Prevalence of resistance MIC	Prevalence of carriage, prevalence of resistance, and MIC ranges 48 months after MDA	<u>Prevalence of carriage (treated)</u> 48 months: 13% (138/1047) <u>Prevalence of resistance (treated)</u> 48 months: 29% (40/138) <u>Prevalence of MICs ≥8 µg/mL</u> 48 months: 80% (32/40)	The authors found a moderate amount of resistance in 4 years after the mass distribution of azithromycin

MBC = Minimum Bactericidal Concentration, MCC = Minimum Chlamydicidal Concentration, MIC =Minimum inhibitory concentration, NR = Not Reported
*Unless otherwise indicated, reported as % prevalence (number of isolates identified with carriage or resistance/total number of samples)
†Same study population

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1.8 Supplemental Material

Supplemental Table 1. Risk of bias in randomized and non-randomized studies on macrolide resistance after mass azithromycin for trachoma in multiple organisms.*

Study	Confounding	Selection Bias			Misclassification Bias	Performance Bias		Detection Bias	Attrition Bias	Reporting Bias
	Bias due to confounding (O)	Selection into study (O)	Random sequence generation (R)	Allocation concealment (R)	Bias in intervention classification (O)	Bias due to deviations from interventions (O)	Masking of participants and personnel (R)	Masking of outcome assessors (R, O)	Incomplete outcome data (R, O)	Selective reporting (R, O)
Streptococcus pneumoniae										
Randomized controlled trial										
Skalet, 2010(7)	NA	NA	Low	Low	NA	NA	Low	Low	Low	Low
Keenan, 2018(11)	NA	NA	Low	Low	NA	NA	Low	Low	Low	Low
Longitudinal (cohort and single group)										
Leach, 1997(1)	High	Unclear	NA	NA	Unclear	Unclear	NA	Unclear	Unclear	Unclear
Coles, 2013(8)	Moderate	Low	NA	NA	Low	Low	NA	Unclear	Unclear	Unclear
Repeated cross-sectional										
Fry, 2002(2)	Unclear	Low	NA	NA	Low	Unclear	NA	Unclear	Low	Unclear
Batt, 2003(4)	High	Low	NA	NA	Low	Low	NA	Unclear	Low	Unclear
Haug, 2010(6)	Unclear	Unclear	NA	NA	Low	Low	NA	Low	Low	Unclear
Burr, 2014(9)	Moderate	Unclear	NA	NA	Low	Low	NA	Unclear	Low	Unclear

Cross-sectional										
Gaynor, 2003(3)	High	High	NA	NA	Low	Unclear	NA	Unclear	Unclear	Unclear
Gaynor, 2005(5)	Unclear	Unclear	NA	NA	Low	Low	NA	Unclear	Unclear	Unclear
Bloch, 2017(10)	High	Low	NA	NA	Low	Unclear	NA	Unclear	Unclear	Unclear
Lietman, NA(12)	High	Low	NA	NA	Low	Low	NA	Low	Low	Unclear
Chlamydia trachomatis										
Longitudinal (cohort and single group)										
Solomon, 2005(13)	High	Low	NA	NA	Low	Low	NA	Unclear	Low	Unclear
West, 2014(15)	High	Unclear	NA	NA	Low	Unclear	NA	Unclear	Unclear	Unclear
Cross-sectional										
Hong, 2009(14)	Unclear	Low	NA	NA	Low	Unclear	NA	Low	Unclear	Unclear
Escherichia coli										
Longitudinal (cohort and single group)										
Seidman, 2014(16)	Moderate	Low	NA	NA	Low	Low	NA	Unclear	Unclear	Unclear
Seidman, 2016(17)	Moderate	Low	NA	NA	Low	Low	NA	Unclear	Unclear	Unclear

Cross-sectional										
Bloch, 2017(10)	High	Low	NA	NA	Low	Unclear	NA	Unclear	Unclear	Unclear
Plasmodium falciparum										
Longitudinal (cohort and single group)										
Schachterle, 2014(18)	Moderate	Low	NA	NA	Low	Low	NA	Unclear	Unclear	Unclear
Staphylococcus aureus										
Repeated cross-sectional										
Bojang, 2017[79]	Unclear	Unclear	NA	NA	Low	Low	NA	Unclear	Low	Unclear
Cross-sectional										
Bloch, 2017(10)	High	Low	NA	NA	Low	Unclear	NA	Unclear	Unclear	Unclear

O=domain defined for observational, non-randomized studies; R=domain defined for randomized studies
*Assessed using the Cochrane Collaboration's risk of bias tool for randomized interventions and Cochrane's ROBINS-I tool for non-randomized interventions

2. Chapter 2. Biannual azithromycin distribution and child mortality among malnourished children: a subgroup analysis of a cluster-randomized trial

2.1 Abstract

Biannual azithromycin distribution has been shown to reduce child mortality as well as increase antimicrobial resistance. Targeting distributions to vulnerable subgroups like malnourished children is one approach to reaching those at the highest risk of mortality while limiting selection for resistance. The objective of this analysis was to assess whether the effect of azithromycin on mortality differs by nutritional status. A large simple trial randomized communities in Niger to receive biannual distributions of azithromycin or placebo to children 1-59 months old over a 2-year timeframe. In subgroup analyses, the effect of azithromycin distribution on child mortality was assessed for underweight subgroups using weight-for-age Z-score (WAZ) thresholds of -2 and -3. Modification of the effect of azithromycin on mortality by underweight status was examined on the additive and multiplicative scale. Of 27,222 children 1-11 months of age from 593 communities who had weight measured at their first entry into the study, approximately 23% had a WAZ < -2 and 10% had a WAZ < -3. The mortality rate was lower in azithromycin communities than placebo communities overall, with larger reductions among children with lower WAZ. The mortality rate difference comparing azithromycin to placebo communities was -12.6 deaths per 1,000 person-years (95% CI -18.5 to -6.9) overall, -17.0 (95% CI -28.0 to -7.0) among children with WAZ < -2, and -25.6 (95% CI -42.6 to -9.6) among children with WAZ < -3. No statistically significant evidence of effect modification was demonstrated by WAZ subgroup on either the additive or multiplicative scale. The estimated number of deaths averted with azithromycin distribution was 388 (95% CI 215 to 573) overall, 116 (95% CI 48 to 192) among children with WAZ < -2, and 76 (95% CI 27 to 127) among children with WAZ < -3. Although mortality rates were higher in the underweight subgroups, this study was unable to demonstrate that nutritional status modified the effect of biannual azithromycin distribution on mortality. Even if the effect were greater among underweight children, a non-targeted intervention would result in the greatest absolute impact on number of deaths averted.

2.2 Introduction

Biannual azithromycin distribution reduced mortality among children 1-59 months of age in a large cluster-randomized trial in Malawi, Niger, and Tanzania (MORDOR trial, Macrolides Oraux pour Réduire les Décès avec un Oeil sur la Résistance).[29,30] In conjunction with existing child survival activities, this intervention has the potential to bolster progress in reducing under-5 mortality, particularly in high mortality settings. However, these distributions increase the prevalence of antimicrobial resistance.[42,103] Limiting antibiotic distributions to smaller subgroups at the highest risk of mortality might be an approach to reduce selection for resistance.[104]

Malnutrition is implicated in up to 45% of all childhood deaths globally.[43] Malnourished children are at increased risk of mortality from infectious diseases such as diarrhea and respiratory tract infections.[43] Moreover, the relationship between malnutrition and infection is complex, with undernutrition suppressing the immune system and increasing the risk of infection, and infection causing a reduction in appetite, malabsorption of nutrients, and competition for nutrients.[105,106] Provision of antibiotics to malnourished children could lead to clearance of both overt and

subclinical infections associated mortality. Use of antibiotics with a long half-life, like azithromycin, could also prevent the development of infections during the 1-2 weeks after administration.[107] Other proposed mechanisms for a beneficial effect of antibiotics in undernourished children involve modulation of the intestinal microbiota, which could result in a reduction in gut flora that compete for nutrients and affect chronic conditions like environmental enteropathy.[106,108-112]

Multiple studies have examined the role of antibiotics in malnourished children, with varying results. Three individual-randomized trials have compared antibiotics to placebo in the management of severe acute malnutrition.[45,46,110] One trial in Malawi found that children receiving antibiotics experienced greater nutritional recovery and less mortality than those receiving placebo,[45] whereas two other trials found no difference in either nutritional recovery or mortality between arms.[46,110] Fewer studies have focused on children with moderate malnutrition, although one multi-country trial evaluating the effect of antibiotics on a number of outcomes in children with moderate acute malnutrition is currently underway.[113]

Targeting azithromycin to high risk subgroups like malnourished children could preserve resources and lower the risk of selecting for antimicrobial resistance. However, evidence on the effect of antibiotics on mortality in malnourished children is mixed. The MORDOR trial provides an opportunity to examine the role of antibiotics in reducing mortality in malnourished children in a sub-Saharan African setting. The objective of this pre-specified subgroup analysis was to assess whether the effect of biannual distribution of oral azithromycin on child mortality differed by nutritional status in Niger.

2.3 Methods

Trial Design, Setting, and Participants

MORDOR was a large simple multi-site cluster-randomized trial designed to compare the effect of biannual distribution of oral azithromycin to placebo on child mortality.[29] This analysis included the Niger site, which enrolled communities in the Boboye and Loga districts (now Boboye, Loga, and Falmey districts after nation-wide redistricting). Communities with populations between 200 and 2,000 inhabitants according to the Niger 2012 census were eligible for inclusion in the main trial. Children 1-59 months of age who weighed ≥ 3.8 kg were eligible for treatment. This subgroup analysis included children 1-11 months old who had weight recorded at the time of the child's first census, regardless of census phase. Children 12-59 months old were excluded because crude height intervals were used to determine dose in children able to stand, and nutritional status indicators could not be accurately calculated for this group.

Ethical approval for the Niger site was obtained from the Niger Ministry of Health and the University of California, San Francisco Committee on Human Research. Verbal informed consent was obtained from households and caregivers before inclusion. The trial was conducted in accordance with the principles of the Declaration of Helsinki and was registered at clinicaltrials.gov (NCT02047981).

Census

A door-to-door census was conducted every 6 months to enumerate households in the study area between December 2014 and August 2017. Demographic information (age, sex) was recorded for each child 1-59 months old. During follow-up census data collection, vital status (alive, dead, unknown) and residence (living in community, moved outside community, or unknown) were recorded. Five censuses (four inter-census phases) were completed during the 2-year study. Data were collected electronically using a custom-designed mobile application (Conexus, Inc.) and uploaded to Salesforce.com.

Interventions

At every biannual census, each child 1-59 months old was offered a single, directly observed dose of oral azithromycin or placebo (Pfizer, Inc., New York, NY). Children were given a dose of 20 mg per kg, which was assessed by height-stick approximation according to Niger's trachoma program guidelines or by weight for children unable to stand. Children known to be allergic to macrolides were not treated. Adverse events were monitored and have been reported elsewhere.[40,53]

Outcomes

The outcome for this analysis is mortality, defined as community mortality rate (deaths per 1,000 person-years at risk). Data collected during the biannual census were used to assess the outcome. A death was included if a child was recorded as alive on one census and died while living in the community at the subsequent census. Person-time at risk was calculated as the number of days between consecutive census periods or until death. Children who moved or had an unknown status at the subsequent census contributed half of the days during that inter-census period.

Assessment of Nutritional Status

The trial protocol included assessment of weight for the purpose of determining dosage in children unable to stand. Study personnel recorded weight (if measured) and dose administered for all children in the mobile application. To determine dosage, children unable to stand were weighed once at each visit (Amw-tl440 digital hanging scale, American Weigh Scales, Georgia, USA) and weight was recorded to the nearest 0.1 kg. Age- and sex-adjusted weight-for-age Z-scores (WAZ) were calculated using the 2006 WHO Child Growth Standards with the zscorer package in R (R Foundation for Statistical Computing, Vienna, Austria).[114-116] WAZ was dichotomized to group children without or with moderate to severe malnutrition ($WAZ \geq -2$ and $WAZ < -2$ and, respectively) and without or with severe malnutrition ($WAZ \geq -3$ and $WAZ < -3$ and, respectively). These categories were chosen to align with current classification standards used in nutritional policies and programs. Children with a baseline WAZ of less than -6 or greater than 5 were excluded according to WHO recommendations.[115]

Randomization and Masking

Within each country, communities were randomized 1:1 to receive biannual azithromycin or placebo. The randomization sequence was generated in R by the trial biostatistician and was implemented by unmasked members of the data team and Pfizer. The allocation was concealed by simultaneous randomization assignment. Participants, investigators, data collectors, and data analysts were masked to treatment assignment. Placebo was packaged to be identical in appearance to the azithromycin to maintain masking.

Sample Size and Statistical Methods

The MORDOR trial was designed and powered for the primary outcome, which been previously published.[53] Briefly, the overall trial had 80% power to detect a 10% difference in all-cause mortality among communities receiving azithromycin compared to placebo, and the main trial in Niger included 594 eligible communities.[53] Given the fixed design, the prevalence of underweight, and the mortality rates within subgroups, this subgroup analysis had 80% power to detect additive interaction effects of the following sizes, interpreted as the mortality rate among underweight children receiving placebo in excess of the individual effects of underweight or placebo on mortality: 17 deaths per 1,000 person-years for the moderate to severe subgroup and 25 deaths per 1,000 person-years for the severe subgroup.[117]

Analyses were conducted in R. Participant characteristics, WAZ, and outcomes were summarized by arm using frequency and percentage for categorical variables, mean and standard deviation (SD) for continuous variables, and incidence rate (deaths per 1,000 person-years, hereafter referred to as "mortality rate") and 95% confidence interval (CI) for outcomes. Confidence intervals were constructed using percentiles from bootstrap resampling with 1,000 replicates. Participant characteristics were also compared among those included in the analysis and those excluded for having missing or invalid weight measurements. No multiple comparisons corrections were made.

Effect modification was evaluated non-parametrically with interaction contrasts.[118] To calculate the contrasts, subgroups were coded such that the groups with the lowest mortality rates were the reference categories (i.e., R_{00} = mortality rate among higher weight children in azithromycin communities, R_{01} = mortality rate among underweight in azithromycin communities, R_{10} = mortality rate among higher weight children in placebo communities, and R_{11} = mortality rate among underweight children in placebo communities).[119] An additive interaction contrast greater than 0 indicates the joint effect of receiving placebo and being underweight is greater than the sum of the individual effects considered separately. A multiplicative interaction contrast greater than 1 indicates the joint effect of receiving placebo and being underweight is greater than the product of the individual effects considered separately. The absolute number of deaths averted with azithromycin in each subgroup was also estimated using person-time at risk in both arms and the subgroup-level mortality rates.

Several sensitivity analyses were conducted. Survival probability was summarized by treatment arm and WAZ subgroup using Kaplan-Meier survival curves. Effect modification was also examined using Cox proportional hazards models. To determine the presence of multiplicative

interaction, models included a shared frailty assuming a gamma distribution to account for clustering, the Efron method for ties, and treatment and WAZ as covariates with their product as an interaction term. Model estimates were reported with hazard ratios for each subgroup against a single reference category and with hazard ratios for the effect of treatment within each stratum of WAZ.[118,120] The estimated hazard ratios were used to calculate the Relative Excess Risk due to Interaction ($RERI_{HR}$) to assess the presence and direction of additive interaction, with the same coding as used for the interaction contrasts.[118-121] The delta method was used to calculate standard errors for the $RERI_{HR}$.[118] As treatment arm was randomized and is the primary intervention of interest, confounding of the relationship between nutritional status and mortality was not considered and no additional factors were controlled for in the models.[118] Model assumptions were evaluated graphically with ln(-ln) survival plots and analytically with tests of scaled Schoenfeld residuals as well as with models including terms for interactions with time to event for each covariate. The appropriateness of the distributional assumptions for the shared frailty were assessed using a lognormal distribution in the frailty models and by using generalized estimating equations (GEE) to account for clustering.

Additional sensitivity analyses included evaluating the potential for bias induced by the selection of the analysis sample by restricting the analysis to children eligible during the first phase only and by restricting to children 1-5 months of age. To assess the impact of the use and form of WAZ, baseline weight, age, and sex were included in the models and baseline WAZ was assessed in continuous form. To evaluate assumptions made in determining time to mortality when no exact date was available, an interval censoring method was also used. This was implemented as a generalized linear mixed model, with a binary outcome for death, a complementary log-log link, and a term for census phase.

2.4 Results

In December 2014, 615 communities in Niger were randomized to receive biannual azithromycin or placebo in the main trial, of which 594 communities were successfully censused and included in analyses (Figure 1). Treatment coverage among children 1-59 months old was greater than 91% over the four treatment phases in both arms. The final sample for this analysis included 593 communities with 27,222 children 1-11 months old who had a valid weight recorded at the time of the child's first entry into the study. One community was not included because it had no eligible children, and 12,086 children 1-11 months old at their first census were excluded either for having no weight recorded (11,899 children, of which 10,271 had approximate height measured) or having a WAZ less than -6 or greater than 5 recorded (187 children). Over the 2-year study period, 4,921 children were lost to follow-up, with a similar percentage of children lost in each arm (Supplemental Table 1).

Characteristics of included children at the time of the child's first census are shown by treatment arm in Table 1. Overall, the median age was 4 months (IQR 3 to 6) and 49.5% of children (13,484/27,222) were female. Mean WAZ was -0.8 (SD 1.7), with 23.0% (6,268/27,222) of all children having a WAZ < -2 and 10.1% (2,755/27,222) having a WAZ < -3. All characteristics were similar in both arms. Excluded children were older than included children (median age 9 months, IQR 6-11) and a similar percentage were female (49.1%, Supplemental Table 2).

The analysis included 1,184 deaths and a total of 30,852 person-years at risk. The overall difference in the incidence of mortality comparing azithromycin communities to placebo communities was -12.6 deaths per 1,000 person-years (95% CI -18.5 to -6.9; Table 2). By subgroup, this difference was -17.0 (95% CI -28.0 to -7.0) among those with WAZ < -2, and -25.6 (95% CI -42.6 to -9.6) among those with WAZ < -3. Figure 2 compares mortality rates by treatment arm and subgroup. Interaction contrasts on the additive scale were 5.7 deaths per 1,000 person-years (95% CI -6.4 to 16.8) for the moderate to severe subgroup and 14.4 deaths per 1,000 person-years (95% CI -2.2 to 31.1) for the severe subgroup. On the multiplicative scale, these contrasts were 1.1 (95% CI 0.8 to 1.4) and 1.2 (95% CI 0.9 to 1.7), respectively. The estimated number of deaths averted with azithromycin among children 1-11 months old was 388 (95% CI 214 to 574) overall, 116 (95% CI 48 to 192) among children with WAZ < -2, and 76 (95% CI 27 to 127) among children with WAZ < -3.

Figure 3 displays survival probabilities by arm and subgroup and Table 3 reports model-based estimates of mortality and effect modification by subgroup. Among children in placebo-treated communities, lower WAZ was associated with an increased hazard of mortality (HR 1.32, 95% CI 1.11 to 1.57 comparing WAZ < -2 to WAZ ≥ -2 and HR 1.56, 95% CI 1.25 to 1.95 comparing WAZ < -3 to WAZ ≥ -3). The hazard for mortality was lower in azithromycin communities than placebo communities, with a more pronounced effect for the subgroups of underweight children (27% lower in WAZ ≥ -2, 95% CI 15 to 38; 30% lower in WAZ < -2, 95% CI 11 to 45; and 38% lower in WAZ < -3, 95% CI 14 to 55). When comparing underweight children in azithromycin communities to higher weight children in placebo-treated communities, the hazards for mortality were similar in both subgroups (HR 0.93, 95% CI 0.75 to 1.14 comparing WAZ < -2 to WAZ ≥ -2 and HR 0.97, 95% CI 0.74 to 1.27 comparing WAZ < -3 to WAZ ≥ -3). No evidence of effect modification was identified. Similar results were found in all sensitivity analyses (Supplemental Table 2).

2.5 Discussion

This subgroup analysis evaluated whether the effect of biannual azithromycin distribution on child mortality differed by underweight status in a high mortality West African setting. Azithromycin was associated with an overall 28% reduction in mortality compared to placebo in children 1-11 months old with weight measured, similar to the age-based subgroup results from the main trial.[29] As expected given evidence on the relationship between malnutrition and mortality,[43,122,123] lower weight-for-age was associated with increased mortality. The observed time to mortality in underweight children receiving azithromycin was approximately the same as that of higher weight children receiving placebo. Although the absolute reduction in mortality between arms appears larger in both underweight groups, no evidence of effect modification by WAZ subgroup was found at the 95% confidence level. The number of deaths averted was greatest if all children were treated with azithromycin, regardless of nutritional status.

The non-specific distribution of azithromycin to reduce child mortality presents an ethical dilemma:[51] given the strong evidence of efficacy, it may be unethical to withhold such an intervention, yet the intervention's effect on antimicrobial resistance warrants caution. Increasing resistance could reduce the efficacy of essential antibiotics, potentially causing additional morbidity and mortality in the longer term. Targeting the intervention to high risk subgroups is

one solution to preserve resources and reduce negative consequences; targeting all children 1-11 months in this study population required 10 times the amount of azithromycin compared to targeting WAZ < -3. A targeted approach may also be more cost-effective than a broader distribution strategy.[124] The assumption that targeting vulnerable subgroups results in the greatest population health benefits has been questioned, however, since more lives are saved by intervening on a population with a wider risk spectrum.[125-127] Here, although there is some indication that intervening on those with the lowest WAZ may be particularly beneficial, the absolute number of deaths averted was 5 times greater when including all children 1-11 months as opposed to only the 10% with WAZ < -3. In addition, if indirect effects of the intervention are present, these might be lost with a more focused intervention. Finally, targeting a subgroup of the population presents its own ethical complexity, as providing a beneficial intervention more broadly might be more equitable when resources are available to do so.[51]

Approximately 23% of the children included in this analysis were underweight, similar to other estimates indicating that Niger bears a high burden of malnutrition.[128] A single weight measurement was taken on a subset of children 1-11 months old who were unable to stand, which has several implications for interpretation of these results. First, other nutritional status indicators like wasting and stunting could not be assessed. Underweight status has been shown to increase the risk of mortality in multiple settings,[43,122,123,129,130] with some evidence demonstrating that WAZ alone is a highly sensitive and specific indicator of concurrent wasting and stunting.[131] Second, the selection of children 1-11 months of age who had weight measurements available could induce bias, since children at the older end of that range able to stand were more likely not to be weighed. However, exclusions among the older age group were balanced by arm, overall and across phases, and sensitivity analyses restricting the population to children 1-5 months produced similar results to the main analysis. The analysis population might not be representative of the general population, as it might include a higher prevalence of underweight children and does not reflect the experience of children 12-59 months old. Third, the SD for WAZ was greater than 1,[115] likely due to measurement error since weight was assessed primarily for the purpose of intervention delivery. Only one measurement was taken at each visit in order to determine dosage. As mean WAZ and SD were similar across arms, any information bias is likely to be conservative, which could have masked the presence of effect modification. Fourth, the prevalence of malnutrition is known to vary with seasonal food insecurity in West Africa.[132] Seasonality-focused analyses were not pursued given the low power to further stratify the population, and the lack of an overall seasonal effect of azithromycin on mortality in the main trial.[133] Finally, the use of cutoffs to categorize malnourished groups has been criticized for creating false separation of subgroups in which to intervene,[134] particularly in high burden areas where the entire distribution of anthropometric indicators is shifted downwards. As these cutoffs are actively used in current programs and policy, their use in this application provides readily available information to these sectors, while also calling into question the impact of a targeted strategy that would exclude many children with mild to moderate malnutrition who also face an increased burden of mortality.[122]

Additional limitations of this study include those shared by most subgroup analyses of trials, such as the potential for false negatives from lack of power and bias from use of improper subgroups. The effect sizes observed in this analysis were smaller than detectable by the design (5.7 vs 17 deaths per 1,000 person-years for the moderate to severe subgroup, and 14.4 vs 25

deaths per 1,000 person-years for the severe subgroup), indicating the analysis was underpowered. The use of baseline WAZ from children who entered the study after azithromycin had been distributed at the community level could result in bias since WAZ for these children is a post-randomization characteristic that could be influenced by treatment arm. A sensitivity analysis restricted to the first phase of the study did not reveal differences in results. Also, underweight prevalence did not differ by arm across phases, so more complex approaches to assessing or controlling for this potential bias were not pursued. In this type of dynamic cohort, differential loss to follow-up can result in selection bias. Although loss to follow-up was present, it was similar when compared by arm. Further research would be required to determine whether these results were generalizable to settings beyond those similar to Niger, which has a high burden of both malnutrition and mortality. Strengths of this study include the large sample size, the assessment of both additive and multiplicative interaction, and the randomized design.

2.6 Conclusions

In summary, a placebo-controlled trial found that biannual azithromycin distribution reduced mortality among 1-11-month-old children regardless of underweight status. Although the benefit of azithromycin was greater among subgroups of underweight children, underweight status was not a statistically significant effect modifier in this trial. Treatment of all children 1-11 months old children would save 5 times as many lives as restricting treatments only to children with a WAZ < -3.

2.7 Figures and Tables

Figure 1. CONSORT participant flow diagram. CONSORT, Consolidated Standards of Reporting Trials.

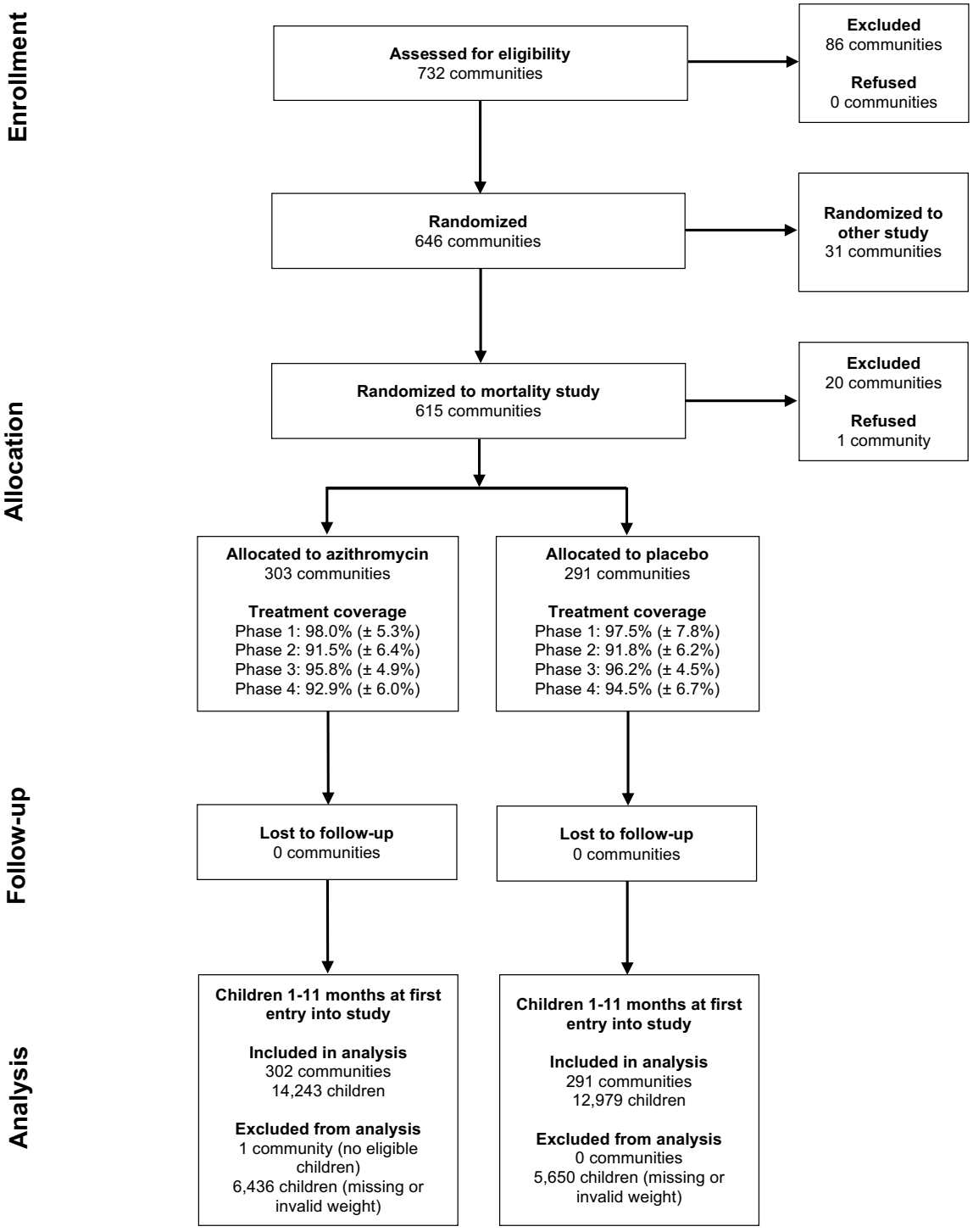


Figure 2. Comparison of mortality rates by treatment arm and WAZ subgroups with interaction contrasts. A/B) Comparisons of mortality rate (deaths per 1,000 person years) by treatment overall and by WAZ subgroup on the additive (A) and multiplicative (B) scales. A) Mortality rate differences (mortality rate in azithromycin communities minus mortality rate in placebo communities). B) Mortality rate ratios (mortality rate in azithromycin communities divided by mortality rate in placebo communities). C/D) Interaction contrasts on the additive (C) and multiplicative (D) scales. Interaction contrasts defined subgroups such that the groups with the lowest mortality rates were the reference categories (i.e., R_{00} = mortality rate among higher weight children in azithromycin communities, R_{01} = mortality rate among underweight in azithromycin communities, R_{10} = mortality rate among higher weight children in placebo communities, and R_{11} = mortality rate among underweight children in placebo communities). C) Interaction contrasts on the additive scale. D) Interaction contrasts on the multiplicative scale.

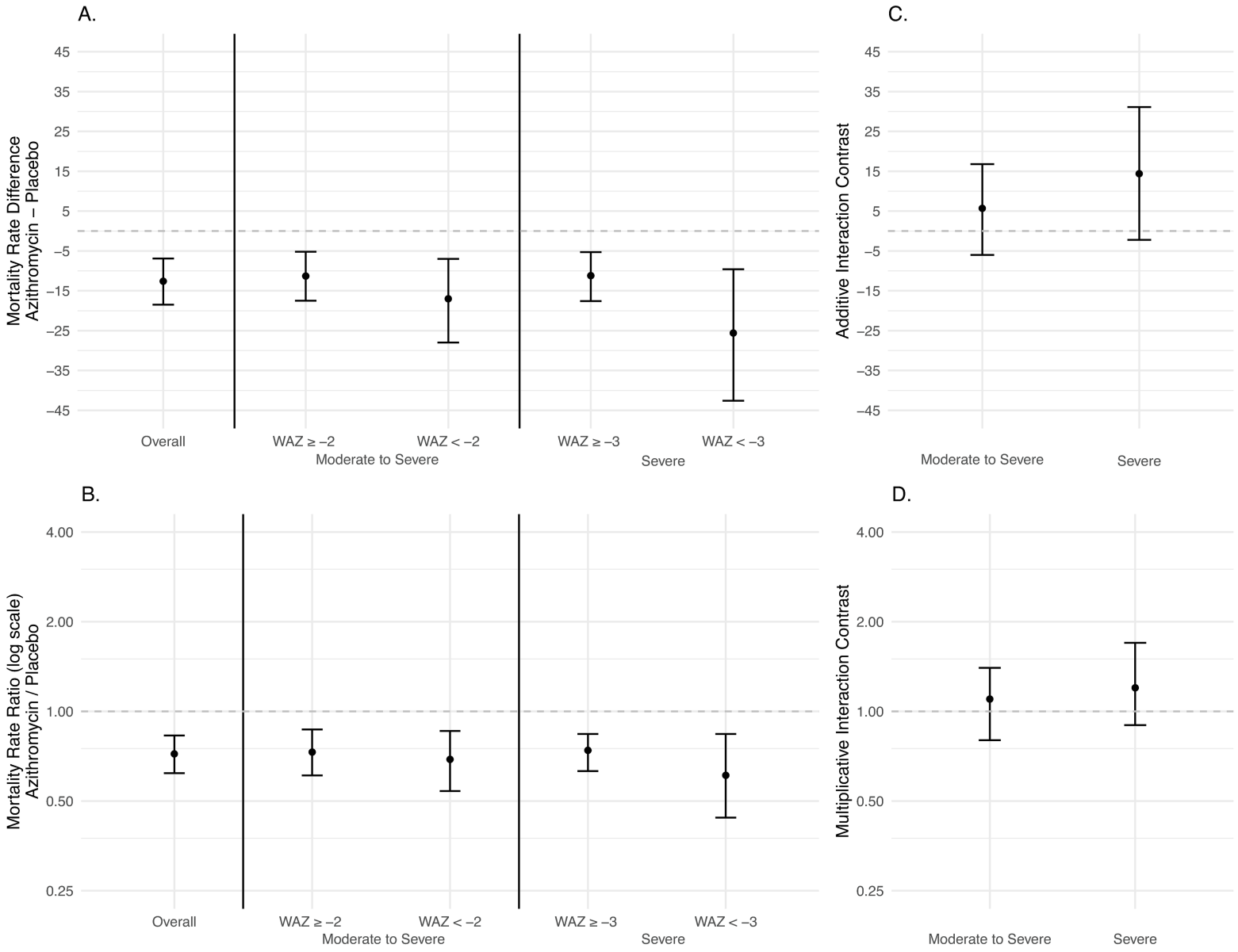
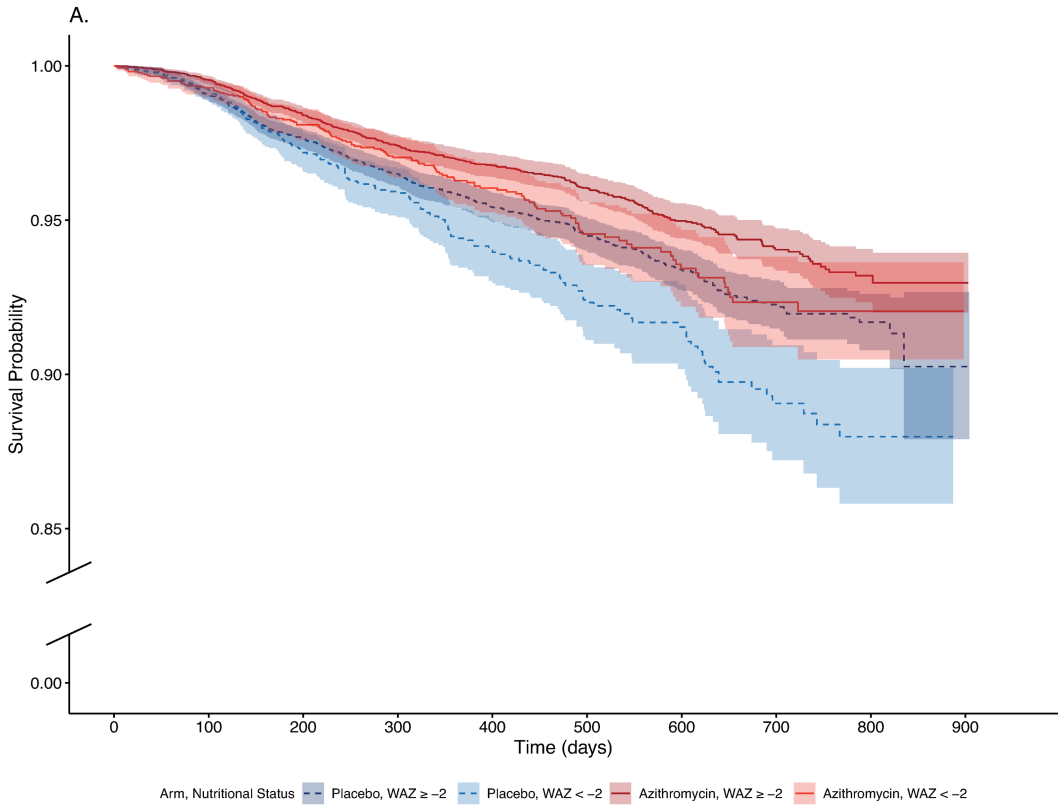

A.
Mortality Rate Difference
Azithromycin – Placebo
45
35
25
15
5
−5
−15
−25
−35
−45
Overall
WAZ ≥ −2
WAZ < −2
WAZ ≥ −3
WAZ < −3
Moderate to Severe
Severe
C.
Additive Interaction Contrast
Moderate to Severe
Severe
B.
Mortality Rate Ratio (log scale)
Azithromycin / Placebo
4.00
2.00
1.00
0.50
0.25
Overall
WAZ ≥ −2
WAZ < −2
WAZ ≥ −3
WAZ < −3
Moderate to Severe
Severe
D.
Multiplicative Interaction Contrast
Moderate to Severe
Severe

Figure 3. Kaplan-Meier survival estimates by treatment arm and WAZ subgroups. Each curve depicts a different subgroup, with placebo represented by dotted lines in shades of blue and azithromycin represented by solid lines in shade of red. The darker shades indicate the higher weight subgroup (WAZ ≥ -2 in A or ≥ -3 in B) and the lighter shades indicate the underweight subgroup (WAZ < -2 in A or < -3 in B). The y-axis is broken for clarity and jumps from 0.00 to 0.85. A) Survival probability by treatment arm and moderate to severe WAZ subgroups. B) Survival probability by treatment arm and severe WAZ subgroups.


A.
1.00
0.95
0.90
0.85
0.00
Survival Probability
0
100
200
300
400
500
600
700
800
900
Time (days)
Arm, Nutritional Status
Placebo, WAZ ≥ −2
Placebo, WAZ < −2
Azithromycin, WAZ ≥ −2
Azithromycin, WAZ < −2

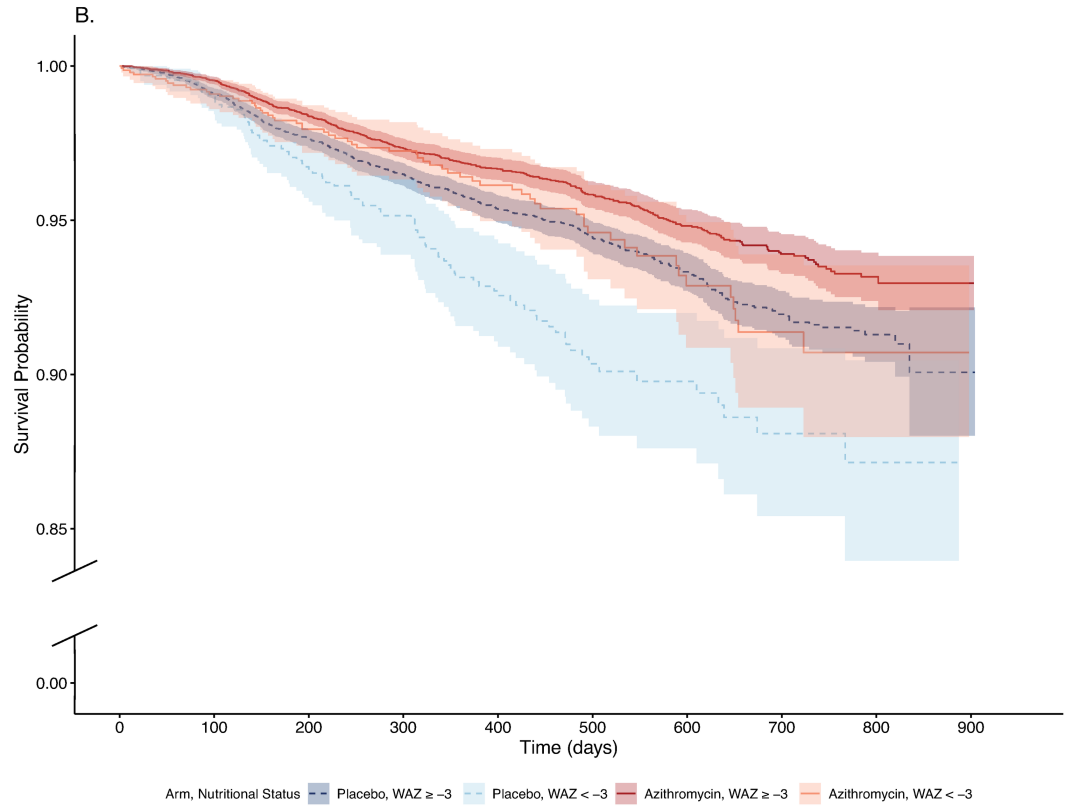

B.
1.00
0.95
0.90
0.85
0.00
Survival Probability
0
100
200
300
400
500
600
700
800
900
Time (days)
Arm, Nutritional Status
Placebo, WAZ ≥ −3
Placebo, WAZ < −3
Azithromycin, WAZ ≥ −3
Azithromycin, WAZ < −3

Table 1. Characteristics of children 1-11 months old with weight recorded at the time of entry into the study.

Characteristic		Azithromycin n = 14,243	Placebo n = 12,979
Age, months, median (IQR)		4 (3-7)	4 (3-6)
Female sex, n (%)		7,040 (49.4%)	6,444 (49.6%)
Phase of entry into study, n (%)			
	1	4,470 (31.1%)	4,034 (31.4%)
	2	3,880 (28.3%)	3,673 (27.2%)
	3	2,751 (20.0%)	2,592 (19.3%)
	4	3,142 (20.6%)	2,680 (22.1%)
WAZ, mean (±SD)		-0.8 (±1.7)	-0.8 (±1.7)
WAZ category, moderate to severe, n (%)			
	≥ -2	10,988 (77.1%)	9,966 (76.8%)
	< -2	3,255 (22.9%)	3,013 (23.2%)
WAZ category, severe, n (%)			
	≥ -3	12,796 (89.8%)	11,671 (89.9%)
	< -3	1,447 (10.2%)	1,308 (10.1%)

IQR, interquartile range; SD, standard deviation; WAZ, weight-for-age Z-score

Table 2. Number of deaths, person-time at risk, and mortality rates by treatment arm and subgroups of WAZ.

Category	Azithromycin				Placebo				Mortality Rate Ratio (95% CI)[2]	Mortality Rate Difference (95% CI)[2]
	n	Deaths	Person-years at risk	Mortality Rate[1] (95% CI)[2]	n	Deaths	Person-years at risk	Mortality Rate[1] (95% CI)[2]		
Overall	14,243	523	16,153	32.4 (29.3, 35.5)	12,979	661	14,699	45.0 (40.3, 49.7)	0.72 (0.62, 0.83)	-12.6 (-18.5, -6.9)
WAZ category, moderate to severe										
≥ -2	10,988	387	12,610	30.7 (27.0, 34.4)	9,966	480	11,435	42.0 (36.9, 47.3)	0.73 (0.61, 0.87)	-11.3 (-17.5, -5.2)
< -2	3,255	136	3,543	38.4 (32.4, 44.9)	3,013	181	3,264	55.4 (46.7, 64.9)	0.69 (0.54, 0.86)	-17.0 (-28.0, -7.0)
WAZ category, severe										
≥ -3	12,796	460	14,599	31.5 (28.3, 35.0)	11,671	568	13,293	42.7 (37.6, 47.6)	0.74 (0.63, 0.84)	-11.2 (-17.6, -5.3)
< -3	1,447	63	1,554	40.5 (30.9, 49.7)	1,308	93	1,406	66.1 (53.7, 79.4)	0.61 (0.44, 0.84)	-25.6 (-42.6, -9.6)

CI, confidence interval; WAZ, weight-for-age Z-score

[1] Deaths per 1,000 person-years at risk

[2] Confidence intervals calculated using bootstrap resampling at the community level to account for clustering; 1000 replicates were used.

Table 3. Sensitivity analysis using Cox proportional hazards regression to evaluate the association between biannual oral azithromycin distribution and mortality by WAZ subgroups.[1]

WAZ Category	Hazard Ratios (95% CI)			Measures of Effect Modification (95% CI)	
	Placebo	Azithromycin	Azithromycin within strata of WAZ	$RERI_{HR}$ (additive)[2]	Ratio of HRs (multiplicative)
Moderate to severe					
≥ -2	1 (ref)	0.73 (0.62, 0.85)	0.73 (0.62, 0.85)	0.17 (-0.20, 0.55)	0.96 (0.74, 1.25)
< -2	1.32 (1.11, 1.57)	0.93 (0.75, 1.14)	0.70 (0.55, 0.89)		
Severe					
≥ -3	1 (ref)	0.74 (0.64, 0.85)	0.74 (0.64, 0.85)	0.45 (-0.11, 1.01)	0.84 (0.59, 1.19)
< -3	1.56 (1.25, 1.95)	0.97 (0.74, 1.27)	0.62 (0.45, 0.86)		

CI, confidence interval; HR, hazard ratio; $RERI_{HR,}$ Relative Excess Risk due to Interaction from hazard ratios, WAZ, weight-for-age Z-score

[1]As treatment arm was randomized and is the primary intervention of interest, confounding of the relationship between nutritional status and mortality was not considered and no additional factors were controlled for in the model.
[2]For this calculation, subgroups were coded so the groups with the lowest mortality rates (azithromycin arm, higher WAZ subgroup) were the reference categories.

2.8 Supplemental Material

Supplemental Table 1. Characteristics of children 1-11 months old at time of entry included in analysis as lost to follow-up.[1]

Characteristic	Lost to follow-up[1]		
	Azithromycin n = 2,610	**Placebo** n = 2,311	**Total** n = 4,921
Age, months, median (IQR)	5 (3-7)	5 (3-7)	5 (3-7)
Female sex, n (%)	1,278 (49.0%)	1,180 (51.1%)	2,458 (49.9%)
WAZ, mean (±SD)	-0.8 (±1.8)	-0.8 (±1.8)	-0.8 (±1.8)
WAZ category, moderate to severe, n (%)			
≥ -2	1,961 (75.1%)	1,777 (76.9%)	3,738 (76.0%)
< -2	649 (24.9%)	534 (23.1%)	1,183 (24.0%)
WAZ category, severe, n (%)			
≥ -3	2,308 (88.4%)	2,074 (89.7%)	4,382 (89.0%)
< -3	302 (11.6%)	237 (10.3%)	539 (11.0%)

IQR, interquartile range; SD, standard deviation; WAZ, weight-for-age Z-score

[1]Children with a vital status of moved or unknown were considered lost to follow-up, with half the time in the inter-census interval allocated as the time at risk contributing to analyses.

Supplemental Table 2. Characteristics of children 1-11 months old at the time of entry among included versus excluded children.[1]

Characteristic	Included in analyses			Excluded from analyses[1]		
	Azithromycin n = 14,243	Placebo n = 12,979	Total n = 27,222	Azithromycin n = 6,436	Placebo n = 5,650	Total n = 12,086
Age, months, median (IQR)	4 (3-7)	4 (3-6)	4 (3-6)	9 (6-11)	9 (6-11)	9 (6-11)
Female sex, n (%)	7,040 (49.4%)	6,444 (49.6%)	13,484 (49.5%)	3,186 (49.5%)	2,744 (48.6%)	5,930 (49.1%)
Phase of entry into study, n (%)						
1	4,470 (31.1%)	4,034 (31.4%)	8,504 (31.2%)	3,699 (57.5%)	3,297 (58.4%)	6,996 (57.9%)
2	3,880 (28.3%)	3,673 (27.2%)	7,553 (27.7%)	1,356 (21.1%)	1,131 (20.0%)	2,487 (20.6%)
3	2,751 (20.0%)	2,592 (19.3%)	5,343 (19.6%)	645 (10.0%)	554 (9.8%)	1,119 (9.9%)
4	3,142 (20.6%)	2,680 (22.1%)	5,822 (21.3%)	736 (11.4%)	668 (11.8%)	1,404 (11.6%)

SD, standard deviation; WAZ, weight-for-age Z-score

[1]Excluded children are those who were 1-11 months old at the time of entry into the study (n = 12,086) and did not have weight measured (n = 11,899) or had an invalid weight recorded (n = 187).

Supplemental Table 3. Sensitivity analyses to evaluate the association between biannual oral azithromycin distribution and mortality by WAZ subgroups.

Analysis	Hazard Ratios (95% CI)	
	WAZ < -2	WAZ < -3
Main Cox model[1]	0.70 (0.55, 0.89)	0.62 (0.45, 0.86)
Adjusted Cox model[2]	0.70 (0.55, 0.89)	0.62 (0.45, 0.86)
Restricted age Cox model[3]	0.71 (0.50, 1.00)	0.56 (0.35, 0.91)
Restricted phase Cox model[4]	0.72 (0.52, 1.01)	0.62 (0.38, 1.01)
Alternative frailty model[5]	0.71 (0.56, 0.90)	0.63 (0.45, 0.87)
GEE model[6]	0.69 (0.55, 86)	0.61 (0.45, 0.84)
GLMM[7]	0.70 (0.55, 0.89)	0.62 (0.44, 0.86)

CI, confidence interval; GEE, generalized estimating equations; GLMM, generalized linear mixed model; HR, hazard ratio; WAZ, weight-for-age Z-score

[1]Cox proportional hazard regression with shared frailty assuming a gamma distribution to account for clustering and treatment arm and WAZ indicator as covariates including all children 1-11 months of age with a valid WAZ, separately for each subgroup
[2]Main Cox model adjusted for age and sex
[3]Main Cox model restricted to eligible children 1-5 months of age
[4]Main Cox model restricted to eligible children enrolled in the first study phase
[5]Main Cox model with shared frailty assuming a log-normal distribution
[6]Generalized estimating equations as an alternative approach to accounting for clustering in the Cox model
[7]Generalized linear mixed model with a binary outcome for death, complementary log-log link, and a term for census phase

3. Chapter 3. Per protocol and spillover effects in a cluster-randomized trial of azithromycin distribution on childhood mortality in Niger.

3.1 Abstract

Biannual distribution of oral azithromycin to children 1-59 months old reduced mortality in that age group by 18% (95% CI 10% to 26%) in the Niger site of the cluster-randomized MORDOR trial. The placebo-controlled design of this trial enabled unbiased assessment of the effect of azithromycin by subgroups based on receipt of the intervention. Here, we compared mortality in azithromycin communities to placebo communities among eligible treated children to determine the efficacy of this intervention in a per protocol analysis and among eligible untreated children to determine the presence of spillover effects from treated to untreated children. In Niger, 594 eligible communities were randomized to biannual distribution of azithromycin or placebo and followed from December 2014 to August 2017. Mean treatment coverage was 90% (standard deviation 10%) across both arms during the two-year study period. In this analysis, 2,581 deaths were included in the treated subgroup and 245 deaths were included in the untreated subgroup. Among treated children, the mortality rate reduction with azithromycin compared to placebo was 20% (95% CI 12% to 28%), with mortality rates of 16.6 deaths per 1,000 person-years in azithromycin communities and 20.9 deaths per 1,000 person-years in placebo communities. Among untreated children, the relative difference in mortality rate in azithromycin communities compared to placebo communities was 9% (95% CI -21% to 31%), with mortality rates of 33.6 deaths per 1,000 person-years in azithromycin communities and 34.4 deaths per 1,000 person-years in placebo communities. In this controlled trial setting, coverage was quite high, which is an important consideration as programs move towards implementation. This analysis suggested both increased efficacy among treated children compared to the intention-to-treat analysis and a small spillover benefit to untreated children, although the analysis was not powered to detect these small effect sizes. Additional analyses will elucidate factors associated with non-participation, which could be used to identify and target vulnerable populations missed in community-based programs.

3.2 Introduction

A large multi-site cluster-randomized trial demonstrated that biannual distribution of oral azithromycin to children 1-59 months reduced mortality in that age group by 14% (95% CI 7% to 20%) in Malawi, Niger, and Tanzania (Macrolides Oraux pour Réduire les Décès avec un Oeil sur la Résistance, MORDOR trial).[29] The largest effect was observed in Niger, which experienced an 18% (95% CI 10% to 26%) reduction in mortality.[29] While other child survival interventions are implemented and health systems are strengthened, this intervention may present an immediate opportunity to effectively and feasibly reduce child mortality in settings like West and Central Africa where under-5 mortality rates remain persistently high.[4,135] Given the risk of this intervention to select for antimicrobial resistance,[42,136] implementers and policy makers are considering targeted approaches to provide azithromycin to smaller, high-risk subgroups. As this intervention moves from controlled trial settings to real world programs, questions remain about implementation and effectiveness in different areas.

Intention-to-treat (ITT) is typically the primary analysis reported for trials because it provides an unbiased estimate of the effect of *treatment assignment*, which is equivalent to the effect of *treatment* with perfect uptake of the intervention.[137] However, ITT analyses might not always capture the full population-level impact of an intervention. With imperfect uptake, interpretation of ITT estimates depends on the trial design, the pattern of non-participation, and the presence of spillover effects.[49,137] In a placebo-controlled trial with imperfect uptake, ITT analyses will underestimate the effect of treatment.[137] Estimates of the per protocol effect, or the effect among those receiving the intervention, are useful for implementers in settings with varying uptake patterns as real-world program settings might experience greater or lower uptake than controlled trial settings, depending on the intervention.[48] However, naïve analyses that fail to properly account for uptake-related confounding or selection bias may yield biased results.[48,137] In addition, ITT will include, but not distinguish, the presence of spillover effects from those who receive the intervention to those who do not and so may over- or under-estimate the effect of the intervention depending on the nature of the spillover.[49] As a result, spillovers are important for understanding the potential difference in magnitude of effect when considering interventions targeted to smaller subgroups of a population compared to the original population of interest. Targeted programs might produce smaller effects if beneficial spillovers are present in the larger population.

Estimating the effect of an intervention on both treated and untreated subgroups provides information complementary to the ITT analysis, which is useful to program implementers evaluating the potential population-level impact of an intervention in settings with different patterns of uptake. The objective of this subgroup analysis of the MORDOR I - Niger trial was to estimate the effect of biannual distribution of azithromycin on mortality among eligible children who received treatment in a per protocol analysis and among eligible children who did not receive treatment in a spillover effect analysis. The placebo-controlled design of the trial enabled unbiased assessment of the effect of this intervention within subgroups based on receipt of the intervention.

3.3 Methods

Trial Design, Setting, and Participants

The MORDOR I trial was a cluster-randomized large simple trial in Malawi, Niger, and Tanzania designed to assess the efficacy of biannual distribution of azithromycin in reducing all-cause mortality in children 1-59 months of age.[29] This subgroup analysis used data from the Niger site, which included communities with a population between 200-2,000 from the Boboye and Loga districts. Children 1-59 months old weighing ≥ 3.8 kg were eligible for the intervention. All children eligible for the intervention in both arms over the two-year study period were eligible for inclusion in this subgroup analysis.

Ethical approval was obtained from the Niger Ministry of Health and the University of California, San Francisco Committee on Human Research. Verbal informed consent was obtained from households and caregivers before commencing study activities. The trial was registered at clinicaltrials.gov (NCT02047981).

Census, Interventions, and Subgroups

Approximately every six months, a door-to-door census was conducted in all study communities to enumerate households and eligible children, provide treatment, and monitor vital status. During the two-year period of the MORDOR I trial, five censuses were conducted to contribute to four census phases (intervals between two censuses). Demographic information was recorded for eligible children. Follow-up census data collection included vital status (alive, dead, moved, unknown). All data were collected electronically using a mobile application designed by Conexus, Inc. and uploaded to Salesforce (Salesforce.com, Inc.).

Each biannual census included distribution of azithromycin or placebo. Each eligible child was offered a single, directly observed dose of oral azithromycin or placebo (Pfizer, Inc., New York, NY) at a dose of 20 mg/kg, determined by weight or height approximation. Census workers recorded treatment administration, which was used to define subgroups of eligible treated and eligible untreated children during each census phase such that a child could contribute to the treated subgroup in one phase and the untreated subgroup in another. Adverse events were recorded and have been previously reported.[29,40]

Outcomes

The outcome for this analysis was all-cause mortality among children 1-59 months, defined as the community-level mortality rate, or community count of deaths per 1,000 person-years at risk. Vital status collected during the census was used to determine the outcome. A child recorded as alive on one census and died on the subsequent census contributed to the numerator of the outcome. Person-time at risk was defined as the number of days alive and living in a study community between census periods. Children who moved, had an unknown status, or died contributed half of the days in the relevant phase. Children with recorded date of death before the beginning of the current phase were excluded.

Randomization and Masking

Communities were randomized in a 1:1 fashion to receive biannual distributions of oral azithromycin or placebo. The trial biostatistician generated the randomization sequence in R (R Foundation for Statistical Computing, Vienna, Austria) and unmasked members of the data team and Pfizer implemented the sequence. Masking was achieved by using placebo, which was identical in appearance and packaging to the azithromycin, and by coding the treatment assignment. Participants, investigators, data collectors, and outcome assessors were masked to treatment assignment.

Sample Size and Statistical Methods

The MORDOR I trial had 80% power to detect a 10% relative difference in all-cause mortality comparing azithromycin-treated communities to placebo-treated communities at an alpha of 0.05 (primary outcome).[29] The Niger site included 594 eligible communities.[29] Given this fixed sample size, this subgroup analysis had 80% power to detect a relative effect of 15% among eligible treated children and a relative effect of 74% among eligible untreated children.

All analyses were conducted for the eligible treated and eligible untreated groups separately. Characteristics of eligible communities and children at the beginning of each census phase were summarized by arm using mean and standard deviation (SD) or frequency and percentage. Participant characteristics were also summarized by arm and inclusion status. Outcomes were summarized as counts of deaths, person-years at risk, and incidence rates (deaths per 1,000 person-years at risk) by arm overall and by phase and age. Negative binomial regression was used to estimate incidence rate ratios (IRRs) comparing azithromycin communities to placebo communities, with the community-level count of deaths as the outcome, community person-time at risk as an offset, and treatment arm as a covariate. Additional analyses included indicators for phase and age, and likelihood ratio tests were used to determine the presence of interaction between treatment arm and these covariates. Several sensitivity analyses were conducted. One set of analyses included all deaths in both subgroups regardless of recorded date, the second updated the phase of death to the prior phase for those children with recorded death dates before the current phase, using treatment status from the prior phase to define subgroups, and another assessed the effect of azithromycin compared to placebo in the untreated subgroup among untreated eligible household contacts of treated children. Analyses were conducted in R. No multiple comparisons corrections were made.

3.4 Results

Before the baseline census, 615 communities in Niger were randomized to azithromycin or placebo and 594 were included in the trial (Figure 1). Census periods began in December 2014, August 2015, February 2016, August 2016, and February 2017 for a total of four census phases. Treatment coverage was greater than 90% across both arms and all phases (Figure 1, Supplemental Figure 1). Treatment coverage was particularly high in the first phase since the denominator for coverage was established at baseline. In this analysis, the treated subgroup contributed 138,210 person-years and the untreated subgroup contributed 7,207 person-years at risk. Of the 3,615 deaths that occurred during the two-year period, 2,949 were among eligible treated children and 666 were among eligible untreated children. Overall, 789 children were excluded for having a date of death recorded as happening before the current census phase, of which 368 were eligible and treated and 421 were eligible and untreated.

Characteristics of communities and participants at the beginning of each phase are summarized by arm and treatment subgroup in Table 1. In Phase 1, 74,131 eligible children were included in the treated subgroup (Table 1a), and 1,922 eligible children were included in the untreated subgroup (Table 1b). More eligible children were included in the untreated subgroup in subsequent phases (6,368 in Phase 2, 2,977 in Phase 3, and 5,460 in Phase 4). In the treated subgroup, 17.8% of children were 1-11 months of age and 48.6% were female across both arms and phases. Similarly, in the untreated subgroup, 15.6% of children were 1-11 months of age and 49.3% were female. Within each subgroup, characteristics were similar in both arms at the beginning of each inter-census period. When comparing included and excluded deaths, age and sex were balanced by arm within inclusion status and across inclusion status overall (Supplemental Table 1). Similar proportions of children were treated by arm within inclusion status, but this differed across inclusion status, with included deaths being more likely to be

treated (91% of included deaths were treated compared to 46% of excluded deaths, Supplemental Table 1).

Table 2 displays deaths, person-time at risk, and mortality rates for each subgroup by arm, age, and phase. Overall, 2,581 deaths were included in the treated subgroup and 245 deaths were included in the untreated subgroup. The incidence of mortality in the placebo arm was 20.9 deaths per 1,000 person-years (95% CI 19.4 to 22.6) in the treated subgroup and 34.4 deaths per 1,000 person-years (95% CI 28.3 to 39.1) in the untreated subgroup. Sensitivity analyses to determine the impact of exclusion criteria found that mortality rates were higher in in both arms and subgroups using different criteria than the main analysis, particularly in the untreated subgroup (Supplemental Table 2a and 2b).

Comparing azithromycin communities to placebo communities, the overall incidence rate ratio was 0.80 (95% CI 0.72 to 0.88, Figure 2a, Supplemental Table 3a) in the treated subgroup and 0.91 (95% CI 0.69 to 1.21, Figure 2b, Supplemental Table 3b) in the untreated subgroup. In the treated subgroup, all arm comparisons by subgroups of phase and age were statistically significant at the 5% level. None of the comparisons for the untreated subgroup were statistically significant. In the treated subgroup, the strongest observed effect was in the 1-5-month age group (IRR 0.70, 95% CI 0.55 to 0.88). No evidence of interaction by phase or age was found in either subgroup. Sensitivity analyses assessing the impact of exclusion criteria and among household contacts found similar results (Supplemental Table 4).

3.5 Discussion

The MORDOR I trial found that biannual distribution of azithromycin to children 1-59 months reduced mortality 18% (95% CI 10% to 26%) in Niger in an ITT analysis.[29] In this subgroup analysis of that trial, the estimated effect among eligible treated children was similar to the ITT effect (20% reduction, 95% CI 12% to 28%), which is expected given the high treatment coverage. Similar to the main ITT analysis,[29] although no evidence of interaction by age was demonstrated, the strongest observed effect among treated children was seen among the youngest age groups, with a 30% reduction among children 1-5 months old and a 23% reduction among children 6-11 months old. The observed per protocol effect overall and in the age-based analyses were each slightly larger than those reported in the primary ITT analyses, although the differences were not statistically significant.[29]

No evidence of a spillover effect from treated to untreated eligible children was demonstrated, although the effect sizes in all spillover analyses were consistent with small effects. Evidence for spillovers tends to be strongest for interventions with pathways involving reduced transmission of infection, such as mass drug administration, which has been associated with strong spillover effects in some settings.[50] Reductions in trachoma prevalence and infection have been reported in untreated groups after mass azithromycin administration.[14,138,139] As the theorized mechanism of effect for azithromycin distribution on mortality involves a reduction in the burden of respiratory infections, diarrhea, and/or malaria,[22,23,31,140] the hypothesis that this intervention produces spillover effects is plausible. With mortality as an outcome, however, this analysis had limited power to detect significant spillovers given the small effect sizes reported. Trachoma programs also distribute azithromycin to entire communities as opposed to children 1-59 months only and

so might have a greater impact on the community burden of disease than more targeted interventions.

The strengths of this study include the randomized, placebo-controlled design, which allows for an unbiased comparison of treatment arms within subgroups of treatment status. In addition, the large sample size allowed for detectable effects in the treated subgroup. Limitations include the lack of variability in cluster-level uptake (Supplemental Figure 1), which precluded the ability to conduct this subgroup analysis at the level of the unit of randomization.[141] Despite the large sample size, the high treatment coverage resulted in relatively few untreated children and so this analysis lacked the power required to detect a small spillover effect. Moreover, as children contributed person-time to each treatment subgroup independently for each phase in this analysis, the spillover effects presented here do not distinguish between spillovers from treated to untreated children and carry-over effects between treatment rounds. If an effect of azithromycin on mortality were identified, possible explanations include a reduction in transmission of infectious diseases associated with mortality from treated to untreated children as well as longer-term benefits experienced among children who were previously treated even if untreated in the current phase. The measurement error present in the recording of the outcome could introduce bias in the estimation of within-subgroup mortality rates. Children recorded as untreated and died in the same phase might have been untreated because they had already died, artificially inflating the estimate of the mortality rate in the untreated group. For this reason, the main analysis excluded deaths with dates before the current phase and sensitivity analyses confirmed this approach was the most conservative in estimating within-subgroup rates (Supplemental Table 3). As the measurement error was the same in both arms, we expect the impact of any bias on the overall estimates of effect to be conservative and sensitivity analyses found similar results to the main analysis (Supplemental Table 4). The generalizability of these results depends on the uptake of the intervention in different settings, as well as baseline mortality rates, which are notably high in this area of the Sahel.[135]

3.6 Conclusions

Overall, this analysis confirmed the mortality reduction with azithromycin in treated children, suggesting increased efficacy among treated children compared to the original intention-to-treat analysis and the possibility of a small spillover effect to untreated children, although this study was not powered to detect effect sizes as small as seen here. Given the high treatment coverage in this trial, the similarity of the per protocol effect to the intention-to-treat effect is expected. As real-world programs may experience lower coverage, the magnitude of the per protocol effect and possible spillover effects are important in determining potential impact of this intervention in different settings. Further analyses will elucidate factors associated with non-participation, which could be used to identify and target vulnerable populations missed in community-based programs.

3.7 Figures and Tables

Figure 1. CONSORT diagram of the flow of communities and participants through each census phase of the two-year MORDOR I trial in Niger. Data presented for Enrollment, Allocation, and Follow-up have been presented elsewhere.[29] Allocation and Follow-up details are presented as community-level mean ± SD. Analysis is shown by treatment status as recorded at each census, with person-time at risk summed across all census phases and summarized as community-level mean ± SD. An individual child may contribute person-time to both treatment statuses across four census phases.

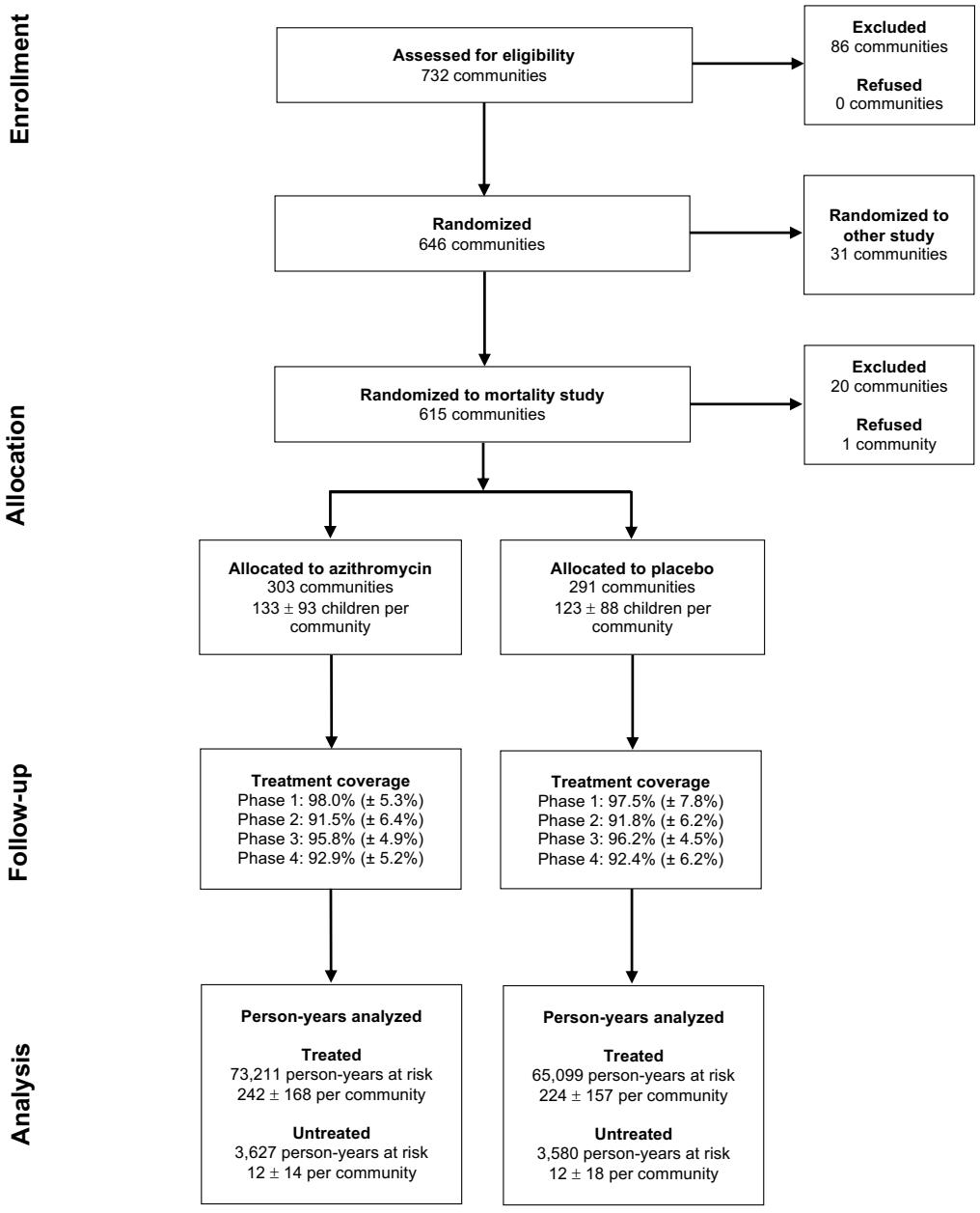


Figure 2. Comparison of mortality rates (deaths per 1,000 person-years at risk) by arm, phase, and age. Incidence rate ratios estimated with negative binomial regression. Figure 2a displays mortality incidence rate ratios for the treated subgroup. Figure 2b displays mortality incidence rate ratios for the untreated subgroup.

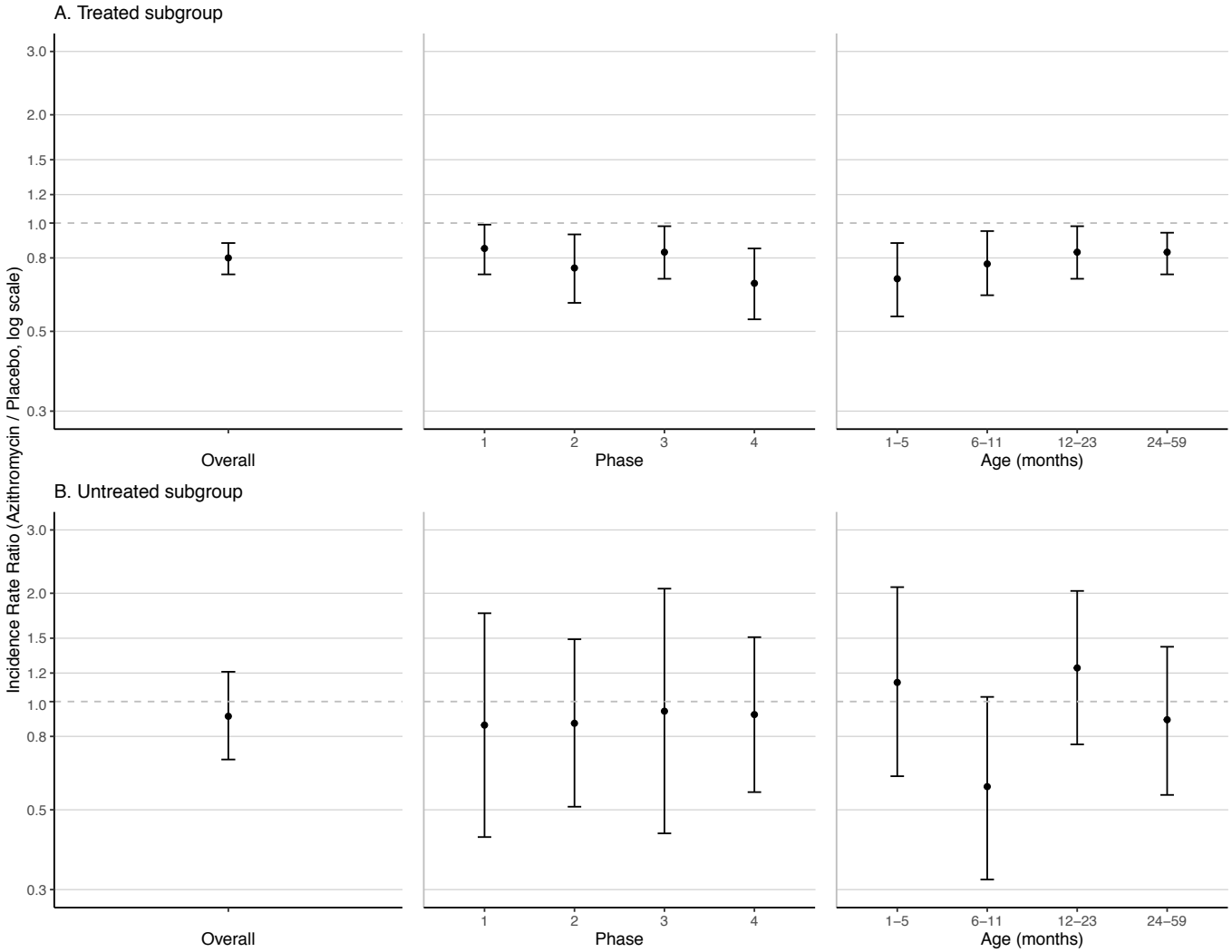


Table 1a. Characteristics of communities and children by study arm and census phase for the treated subgroup.

Characteristic		Phase 1		Phase 2		Phase 3		Phase 4	
		Azithromycin	Placebo	Azithromycin	Placebo	Azithromycin	Placebo	Azithromycin	Placebo
Number of communities		303	291	303	291	303	291	303	291
Number of children		39,414	34,717	37,227	33,520	38,977	34,458	36,574	32,134
Number of children per community, mean (±SD)		130 (±91)	119 (±85)	123 (±86)	115 (±81)	129 (±92)	118 (±82)	121 (±85)	110 (±77)
Age in months, n (%)									
	1-5	2,654 (6.7%)	2,394 (6.9%)	2,575 (6.9%)	2,469 (7.4%)	2,516 (6.5%)	2,286 (6.6%)	3,551 (9.7%)	3,088 (9.6%)
	6-11	5,298 (13.4%)	4,723 (13.6%)	4,224 (11.3%)	3,865 (11.5%)	3,611 (9.3%)	3,242 (9.4%)	2,506 (6.9%)	2,171 (6.8%)
	12-23	6,706 (17.0%)	5,840 (16.8%)	6,985 (18.8%)	6,339 (18.9%)	8,962 (23.0%)	7,939 (23.0%)	8,377 (22.9%)	7,394 (23.0%)
	24-59	24,756 (62.8%)	21,760 (62.7%)	23,443 (63.0%)	20,847 (62.2%)	23,888 (61.3%)	20,991 (60.9%)	22,140 (60.5%)	19,481 (60.6%)
Female, n (%)		19,197 (48.7%)	16,844 (48.5%)	18,226 (49.0%)	16,227 (48.4%)	19,015 (48.8%)	16,669 (48.4%)	17,818 (48.7%)	15,565 (48.4%)

SD, standard deviation

Table 1b. Characteristics of communities and children by study arm and census phase for the untreated subgroup.

Characteristic		Phase 1		Phase 2		Phase 3		Phase 4	
		Azithromycin	Placebo	Azithromycin	Placebo	Azithromycin	Placebo	Azithromycin	Placebo
Number of communities		104	115	289	274	251	229	284	270
Number of children		912	1,010	3,419	2,949	1,556	1,421	2,791	2,669
Number of children per community, mean (±SD)		9 (±18)	9 (±22)	12 (±12)	11 (±12)	6 (±7)	6 (±9)	10 (±11)	10 (±10)
Age in months, n (%)									
	1-5	84 (9.2%)	83 (8.2%)	154 (4.5%)	135 (4.6%)	119 (7.6%)	96 (6.8%)	261 (9.4%)	287 (10.8%)
	6-11	130 (14.3%)	125 (12.4%)	260 (7.6%)	228 (7.7%)	161 (10.3%)	142 (10.0%)	170 (6.1%)	177 (6.6%)
	12-23	135 (14.8%)	169 (16.7%)	428 (12.5%)	320 (10.9%)	332 (21.3%)	311 (21.9%)	649 (23.3%)	597 (22.4%)
	24-59	563 (61.7%)	633 (62.7%)	2,577 (75.4%)	2,266 (76.8%)	944 (60.7%)	872 (61.4%)	1,711 (61.3%)	1,608 (60.2%)
Female, n (%)		479 (52.5%)	528 (52.3%)	1,631 (47.7%)	1,413 (47.9%)	770 (49.5%)	701 (49.3%)	1,414 (50.7%)	1,314 (49.2%)

SD, standard deviation

Table 2a. Incidence of mortality by study arm, age, and phase among the treated subgroup.

Subgroup	Azithromycin			Placebo		
	Deaths	Person-years at risk[1]	Incidence Rate[2] (95% CI)[3]	Deaths	Person-years at risk[1]	Incidence Rate[2] (95% CI)[3]
Overall	1,218	73,211	16.6 (15.3, 17.9)	1,363	65,099	20.9 (19.4, 22.6)
Phase						
1	490	22,790	21.5 (19.0, 23.8)	515	19,885	25.9 (22.9, 28.9)
2	194	15,858	12.2 (10.4, 13.9)	240	14,687	16.3 (13.9, 18.9)
3	362	18,127	20.0 (17.5, 22.3)	385	16,167	23.8 (21.3, 26.6)
4	172	16,437	10.5 (8.5, 12.5)	223	14,360	15.5 (13.3, 17.9)
Age (months)						
1-5	147	5,399	27.2 (22.2, 32.1)	194	4,898	39.6 (33.3, 45.7)
6-11	224	7,604	29.5 (25.3, 33.9)	263	6,851	38.4 (32.7, 45.0)
12-23	330	14,600	22.6 (19.9, 25.3)	354	12,991	27.3 (24.2, 30.5)
24-59	517	45,608	11.3 (10.3, 12.5)	552	40,359	13.7 (12.4, 15.0)

CI, confidence interval

[1]Subgroups might not sum to overall column total due to rounding
[2]Deaths per 1,000 person-years at risk
[3]CIs calculated with bootstrap resampling using 1,000 replicates

Table 2b. Incidence of mortality by study arm, age, and phase among the untreated subgroup.

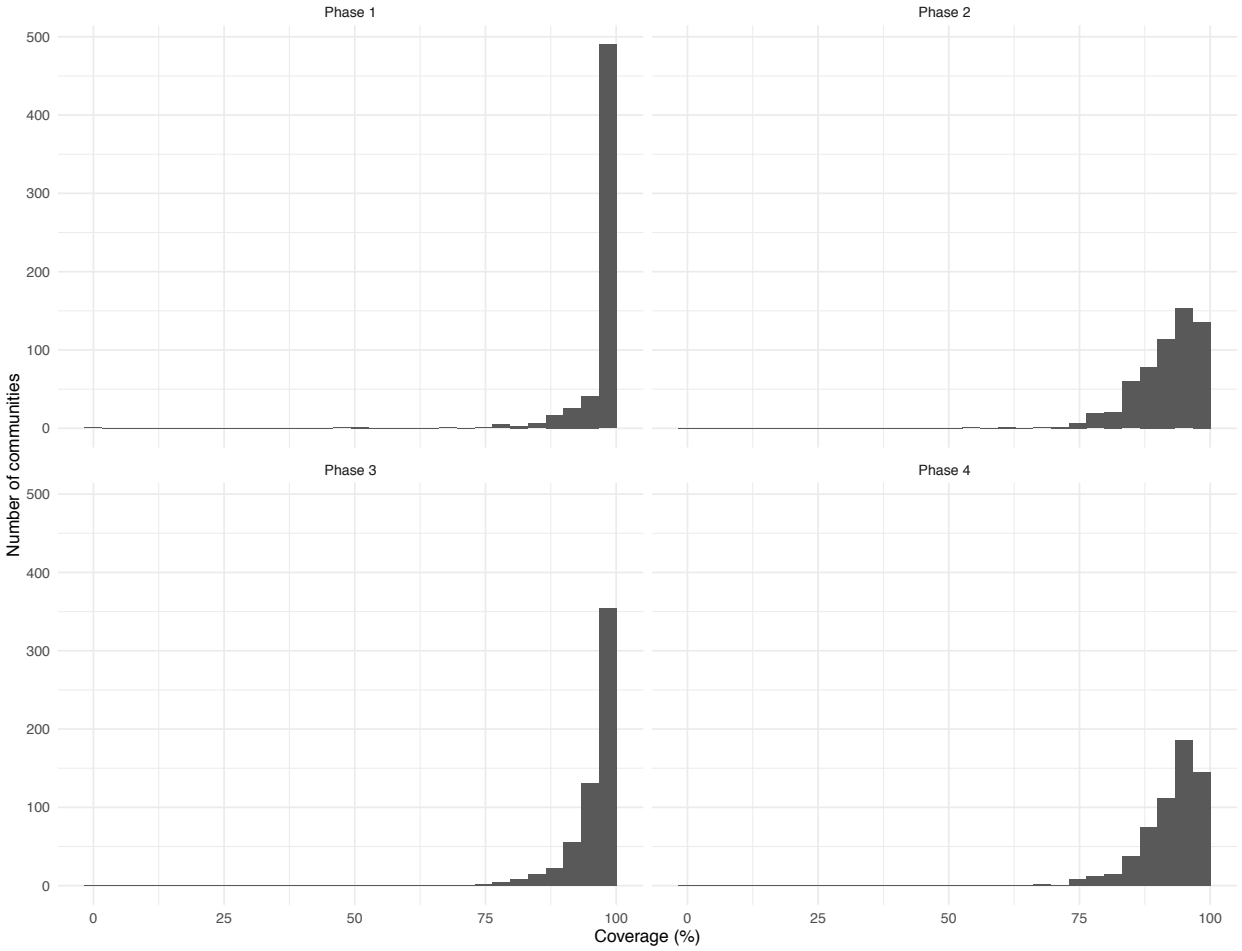
Subgroup	Azithromycin			Placebo		
	Deaths	Person-years at risk[1]	Incidence Rate[2] (95% CI)[3]	Deaths	Person-years at risk[1]	Incidence Rate[2] (95% CI)[3]
Overall	122	3,627	33.6 (27.8, 39.1)	123	3,580	34.4 (28.3, 39.1)
Phase						
1	22	537	41.0 (24.7, 58.4)	22	671	32.8 (16.5, 60.5)
2	32	1,403	22.8 (15.7, 30.0)	32	1,306	24.5 (16.0, 35.9)
3	16	625	25.6 (14.9, 38.4)	15	574	26.1 (12.1, 43.5)
4	52	1,062	49.0 (33.6, 65.5)	54	1,029	52.5 (37.0, 71.4)
Age (months)						
1-5	23	251	91.8 (58.4, 125.7)	20	247	81.0 (49.6, 123.2)
6-11	18	293	61.5 (35.0, 86.8)	30	284	105.5 (70.7, 146.9)
12-23	41	590	69.5 (47.9, 93.6)	31	563	55.1 (36.3, 74.7)
24-59	40	2,493	16.0 (11.4, 21.0)	42	2,486	16.9 (11.4, 23.4)

CI, confidence interval

[1]Subgroups might not sum to overall column total due to rounding
[2]Deaths per 1,000 person-years at risk
[3]CIs calculated with bootstrap resampling using 1,000 replicates

3.8 Supplemental Material

Supplemental Figure 1. Histogram of community-level treatment coverage by study phase. Coverage was defined as the percentage of eligible children with a directly observed administration of azithromycin or placebo per community and phase (n = 594 communities).



Supplemental Table 1. Baseline characteristics among deaths by inclusion status (n = 3,615).

Characteristic		Included (n = 2,826)		Excluded (n = 789)	
		Azithromycin	Placebo	Azithromycin	Placebo
Number of children		1,340	1,486	387	402
Age in months, n (%)					
	1-5	306 (22.8%)	367 (24.7%)	82 (21.2%)	85 (21.1%)
	6-11	335 (25.0%)	377 (25.4%)	106 (27.4%)	113 (28.1%)
	12-23	275 (20.5%)	284 (19.1%)	87 (22.5%)	90 (22.4%)
	24-59	424 (31.6%)	458 (30.8%)	112 (28.9%)	114 (28.4%)
Female, n (%)		667 (48.9%)	740 (49.8%)	181 (46.8%)	201 (50.0%)
Treated,[1] n (%)		1,218 (90.9%)	1,363 (91.7%)	170 (43.9%)	198 (49.2%)

SD, standard deviation

[1]Indicator of whether or not the child was treated in the inter-census period during which death occurred.

Supplemental Table 2a. Comparison of mortality rates by arm in sensitivity analyses among the treated subgroup.

Analysis	Azithromycin			Placebo		
	Deaths	Person-years at risk[1]	Incidence Rate (95% CI)[2]	Deaths	Person-years at risk[1]	Incidence Rate (95% CI)[2]
Primary[3]	1,218	73,211	16.6 (15.7, 17.6)	1,363	65,099	20.9 (19.8, 22.0)
No exclusions[4]	1,388	73,250	18.9 (17.9, 19.9)	1,561	65,144	24.0 (22.9, 25.2)
Re-phase[5]	1,566	73,081	21.4 (20.4, 22.5)	1,729	64,972	26.6 (25.4, 27.9)

Supplemental Table 2b. Comparison of mortality rates by arm in sensitivity analyses among the untreated subgroup.

Analysis	Azithromycin			Placebo		
	Deaths	Person-years at risk[1]	Incidence Rate (95% CI)[2]	Deaths	Person-years at risk[1]	Incidence Rate (95% CI)[2]
Primary[3]	122	3,627	33.6 (28.2, 40.0)	123	3,580	34.4 (28.9, 40.9)
No exclusions[4]	339	3,674	92.3 (83.4, 102.2)	327	3,635	90.2 (81.3, 100.0)
Re-phase[5]	162	3,631	44.6 (38.4, 51.8)	158	3,578	44.2 (38.0, 51.5)
Household contacts[6]	72	1,740	41.4 (33.0, 51.9)	66	1,775	37.2 (29.4, 47.1)

CI, confidence interval

[1]Deaths per 1,000 person-years at risk
[2]Confidence intervals calculated using normal approximation
[3]Primary analysis presented in main text
[4]Sensitivity analysis including all deaths, regardless of recorded death date, with no manipulation
[5]Sensitivity analysis including all deaths, with update of phase of death based on recorded death date
[6]Sensitivy analysis among eligible untreated household contacts of eligible treated children

Supplemental Table 3a. Association between azithromycin distribution and mortality among the treated subgroup by phase and age.

Subgroups	IRR[1]	95% CI
Overall	0.80	0.72, 0.88
Phase		
1	0.85	0.72, 0.99
2	0.75	0.60, 0.93
3	0.83	0.70, 0.98
4	0.68	0.54, 0.85
Age (months)		
1-5	0.70	0.55, 0.88
6-11	0.77	0.63, 0.95
12-23	0.83	0.70, 0.98
24-59	0.83	0.72, 0.94

Supplemental Table 3b. Association between azithromycin distribution and mortality among the untreated subgroup by phase and age.

Subgroups	IRR[1]	95% CI
Overall	0.91	0.69, 1.21
Phase		
1	0.86	0.42, 1.76
2	0.87	0.51, 1.49
3	0.94	0.43, 2.06
4	0.92	0.56, 1.51
Age (months)		
1-5	1.13	0.62, 2.08
6-11	0.58	0.32, 1.03
12-23	1.24	0.76, 2.03
24-59	0.89	0.55, 1.42

CI, confidence interval; IRR, incidence rate ratio

[1]IRRs estimated with negative binomial regression

Supplemental Table 4a. Comparison of the association between azithromycin distribution and mortality in sensitivity analyses among the treated subgroup.

Analysis	IRR[1]	95% CI
Primary[2]	0.80	0.72, 0.88
No exclusions[3]	0.79	0.72, 0.88
Re-phase[4]	0.81	0.73, 0.89

Supplemental Table 4b. Comparison of the association between azithromycin distribution and mortality in sensitivity analyses among the untreated subgroup.

Analysis	IRR[1]	95% CI
Primary[2]	0.91	0.69, 1.21
No exclusions[3]	0.97	0.79, 1.19
Re-phase[4]	0.95	0.74, 1.22
Household contacts[5]	0.96	0.65, 1.41

CI, confidence interval; IRR, incidence rate ratio

[1]IRRs estimated with negative binomial regression
[2]Primary analysis presented in main text
[3]Sensitivity analysis including all deaths, regardless of recorded death date, with no manipulation
[4]Sensitivity analysis including all deaths, with update of phase of death based on recorded death date
[5]Sensitivy analysis among eligible untreated household contacts of eligible treated children

IV. CONCLUSION

In spite of overall progress in reducing mortality globally, under-5 mortality remains persistently high in parts of sub-Saharan Africa. West African countries like Niger bear a particular burden, with up to 10% of children dying before their fifth birthday. Global development agendas like the Sustainable Development Goals include targets for eliminating preventable child mortality. In high mortality settings, highly effective and feasible interventions are urgently needed.

Biannual azithromycin distribution is one intervention with the potential to address the burden of under-5 mortality. This intervention is known to be feasible given the vast global experience in trachoma programs, which have organized the procurement and distribution of more than 900 million doses of azithromycin to communities in trachoma-endemic areas worldwide. Moreover, this intervention has demonstrated efficacy in rigorous controlled trial settings, indicating the potential to prevent nearly 1 in 5 deaths among children in high mortality settings like Niger. While health systems are strengthened to improve support for child survival, this intervention presents an opportunity to reduce under-5 mortality now.

The risk of selection for antimicrobial resistance warrants caution, however, as these distributions could ultimately contribute to unintended increases in morbidity and mortality related to reduced efficacy of macrolides and other antibiotics. In addition, the current evidence for efficacy comes from a controlled trial setting with intensive monitoring and high intervention uptake, which might not mimic real-world conditions and impact. In order to contribute evidence to support decision-making on the use of biannual azithromycin distribution to improve child survival, this dissertation aimed to characterize the risk of antimicrobial resistance more broadly, to understand the effect of the intervention in vulnerable subgroups that might be targeted in a program setting, and to estimate alternative measures of effect to elucidate the full population-level impact of this intervention.

The first chapter examined selection for antimicrobial resistance after mass azithromycin distribution, synthesizing evidence from trachoma programs which have been implementing this intervention for decades. This systematic review identified studies summarizing macrolide resistance after azithromycin distributions in *Chlamydia trachomatis, Streptococcus pneumoniae, Escherichia coli, Staphylococcus aureus,* and *Plasmodium falciparum.* No study found that these distributions increase macrolide resistance in *C. trachomatis,* which is encouraging for trachoma programs as elimination goals rely on continued efficacy of this intervention. Similarly, no evidence demonstrated an effect of azithromycin distribution on macrolide resistance in *P. falciparum.* Multiple studies reported macrolide resistance after azithromycin distributions in *S. pneumoniae, E. coli,* and *S. aureus,* with several suggesting a possible dose-response effect with increasing distribution frequency. The available studies with longer-term follow-up further suggest a decline in resistance towards baseline levels once distributions are stopped, indicating that a short-term intervention may have only short-term effects on resistance. Any research-based or programmatic implementation of this intervention will benefit from inclusion of short- and long-term monitoring for antimicrobial resistance, both during implementation and after distributions are stopped.

The second chapter analyzed the effect of biannual azithromycin on mortality among subgroups defined by underweight status in young children Niger. In this population with a high burden of moderate and severe underweight, underweight status was associated with higher mortality. The observed mortality rate difference in azithromycin compared to placebo communities was larger among underweight children than non-underweight children, but no evidence of effect modification was found. Despite the lack of evidence of a stronger effect among underweight children, however, targeting these distributions to malnourished children still presents an opportunity to limit the amount of antibiotics distributed while focusing the intervention on those at the highest risk of mortality. On the other hand, distributing azithromycin to all children regardless of nutritional status would avert at least 5 times as many deaths as targeting to underweight children. Future research on the impact of targeted distributions on both mortality and antimicrobial resistance will provide an evidence base to complement ethical considerations in refining the delivery approach for this intervention.

The third chapter estimated per protocol and spillover effects of azithromycin distribution on mortality in children in Niger in order to complement the intention-to-treat analysis with program-relevant indicators. The estimated effect among eligible treated children was similar to the intention-to-treat estimate as expected given the high treatment coverage in the trial. No evidence of a spillover effect of azithromycin from treated to untreated children was found, although the observed effect sizes were consistent with small spillovers and this analysis was limited by its small sample size. While this analysis was unable to demonstrate the presence of spillover effects, small effects among untreated children are plausible. At the same time, a lack of spillover effects would suggest that high coverage might be required in programmatic implementation to ensure effects of a similar size as the main trial. The higher mortality rates identified among the untreated children also suggest a vulnerable population with the potential to benefit from inclusion in the intervention, and additional analyses to elucidate factors associated with non-participation could be used to identify and target such groups often missed in community-based programs.

As countries consider the inclusion of biannual azithromycin distribution in child survival programs, questions remain about optimal program design to maximize the mortality benefit while reducing the potential risks. Taken together, this work provides evidence to support implementation-related decision-making as the balance of the benefits are weighed against the risks. Given the likelihood of a short-term increase in antimicrobial resistance, targeted approaches to distribution have the potential to reduce this risk while focusing the intervention's benefit on particularly vulnerable populations. On the other hand, the potential for spillover effects and the 5-fold difference in deaths averted with a non-targeted approach suggests that the greatest population-level impact might come from intervening in a broader risk spectrum. The ethical complexity of this discussion is mitigated by an evidence base to justify implementation choices, motivating continued examination of such choices on both mortality and resistance as well as other child health and broader community outcomes.

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